Identifying the effects of anthropogenic disturbance on the breeding behaviour of female grey seals (Halichoerus grypus), at a mainland UK colony.

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Identifying the effects of anthropogenic disturbance on the breeding behaviour of female grey seals (*Halichoerus grypus*), at a mainland UK colony.

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ABSTRACT

The study aimed to identify whether anthropogenic stimuli constituted a disturbance, as indicated through behavioural responses of the female grey seals (*Halichoerus grypus*) at Donna Nook, a mainland UK breeding colony. The colony has public access to it, and is adjacent to the Ministry of defence training range with frequent fly-overs from low flying aircraft. Data collection was non-intrusive, using in-field focal videos, proximity maps, and human activity data collection. The primary aims of the study were to identify what sources of anthropogenic disturbances, if any, effect grey seal breeding behaviour on the colony. In addition to this the study wished to observe what affect pup sex and pupping location within the colony had on breeding behaviour and individual responses to disturbance. The results of this study were in agreement with prior studies on pinniped species, indicating a very limited behavioural response of individuals to anthropogenic disturbance stimuli during the breeding season. Pedestrian disturbances had a greater impact on the behaviour of individuals than aircraft disturbances; and of all the pedestrian disturbances found at the site, photographers elicited the greatest behavioural response in individuals. Behavioural responses to disturbances were noted to be more significant over the first two minute interval after a disturbance event than over longer periods of time. In vigilance behaviours, consistent individual differences (CIDs) in an individual's response to disturbance events were noted both across AND within disturbance contexts. Pup sex and the location of the birthing site both seemed to affect a female's response to a disturbance event; with mothers of male pups and those females which gave birth close to the Ministry of Defence site showing significantly higher levels of vigilance behaviours after a disturbance event. Comparisons of individual responses to natural and anthropogenic disturbances revealed that individuals show a greater behavioural response to natural disturbances than those disturbances originating from a human source. The lack of behavioural responses to both natural and anthropogenic disturbance sources in the colony indicates the potential role of habituation and/or selection for behavioural types within the colony. The results of this study highlight the scope for future research into the stability of these responses to disturbance stimuli; both over numerous breeding seasons and also in periods outside of the breeding season.

DECLARATION OF COPYRIGHT

I confirm that no part of the material presented in this thesis has previously been submitted by me or any other person for a degree in this or any other university. In all cases, where it is relevant, material from the work of others has been acknowledged.

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1. INTRODUCTION

Although analysis of life-history traits suggest that the age, size and condition of female mammals at parturition will affect both the birth weight, and survival of their offspring (Boltnev and York, 2001); ecological factors such as natural and anthropogenic disturbance events can influence the probability of survival of the offspring to adulthood; by affecting both the size of the mothers at parturition, and the efficiency at which mothers can provision their offspring (Croxall *et al*., 1988). This thesis will aim to examine the effects that anthropogenic disturbance events have on female breeding behaviour at a mainland colony of grey seals (*Halichoerus grypus*) in the UK. This introduction will endeavour to explore the ever expanding research surrounding the different effects that disturbances have on fauna; as well as exploring the ideas of habituation and sensitisation, and the possibility that measurements of behaviour are limited in their reliability of assessing whether a population is responding to a disturbance source.

1.1 Ecotourism

When the concept of 'ecotourism' first began to frequent academic literature in the late 1980s no one could have foreseen the exponential expansion, and prominent position that this ideology would come to hold thirty years later within the tourism sector (Weaver and Lawton, 2007). A report published by the United Nations Environment Convention on Migratory Species (2006) concluded that the demand by people for nature based experiences is growing globally at a faster rate than the general tourism sector (Kirkwood *et al*., 2003; Bejder *et al*., 2006; Weaver and Lawton, 2007). The current global market size of wildlife tourism is a projected twelve million trips per annum; with a 10% growth in the number of trips seen annually (Mintel, 2008; Curtin, 2010). Consumptive wildlife interactions, which involve physical products or materials being removed from the natural environment (artefact collecting, hunting and fishing), have up until now, been the principal focus of wildlife conservationists and academics due to the high publicity they often receive in the media (Loveridge *et al*., 2007; Scarr *et al.,* 2012). However, the increasing effects of non-consumptive wildlife interactions have recently been given growing consideration as a result of the rising human population and exponential rise in the demand for nature based experiences (Woodroffe *et al*., 2005; Christiansen *et al*., 2013). Non consumptive wildlife interactions are those that involve visitor experiences such as bird watching, whale watching, and backpacking. Non-consumptive wildlife interactions do not take anything

physically away from the environment. Although previously seen as less damaging to the environment than consumptive resource use, non-consumptive wildlife interactions can have far reaching negative impacts; both on the focal species itself, and on the wider ecosystem (Green and Higginbottom, 2000). In fact it is widely regarded that in megafauna species the mere presence of human individuals has a direct impact on the megafauna in that area (Reynolds and Braithwaite, 2001).

Conservationists have placed great hopes on ecotourism ‘‘producing economic benefits that encourage conservation’’ (Ryel and Grasse, 1998). Unfortunately there is ample evidence to suggest that ecotourism has the potential to be ecologically unsustainable (e.g. Honey, 1999; Mullner *et al*., 2004). Human disturbance of wildlife is repeatedly cited as one of the key subjects which threaten biodiversity levels in the 21st century (Gill, 2007). The ‘general ecotourist’ market tends to be well-travelled, often with a high level of education, and above average disposable income (Mintel, 2008). They are inclined to avoid areas which are widely frequented by the mass tourism market, and instead pursue an authentic, and to some extent educative experience (Curtin and Wilkes, 2005). While these motivations have resonance with possible sustainable tourism, the literature alludes to the potentially lethal danger to wildlife exposed to wildlife tourism, based upon the assumption that any human presence impacts upon the habitat and its wild inhabitants (Curtin, 2010). For example, within the eco-tourism sector, it has been noted that close encounters with species in their natural habitat is a key determining factor of visitor satisfaction (Wolf and Croft, 2010). Achieving a closer viewing platform is overcome by approaching wildlife but they in turn perceive humans as potential threats, especially in non-captive settings, where irregular visitation and unpredictable behaviour of tourists reduce the likelihood of habituation of individuals (Knight and Gutzwiller, 1995; Wolf and Croft, 2010).

Tourist activities are not always benign; many studies have documented noticeable behavioural and physiological alterations, as well as changes in life-history traits in species as a result of anthropogenic disturbances (Ellison and Cleary, 1977; Cassini, 2001; Walker *et al*., 2006; Scarr *et al.,* 2012; Jiang *et al.,* 2013). For instance, in pinnipeds short-term responses to ecotourism activities include changes in seal vocalisations (Terhune *et al*., 1979), reduction in the time a mother spends with her pup, and an increase in the amount of threat behaviours resulting from a close proximity to tourists (Cassini, 2001). The first response of an individual to a disturbance event which is noticed by scientists in the field is behavioural; this usually manifests itself in an increase in vigilance and/or flight behaviours (Scarpaci *et al*., 2005; Gill,

2007). Secondly, if the disturbance source continues to affect an area for any prolonged length of time, there can be changes in the distribution of a species, by provoking individuals to permanently leave areas subjected to high levels of human activity. As a consequence of these habitat perturbations caused by human disturbance events, some individuals become more susceptible to diseases, while others show indications of a diminished survival and reproductive success rate (Gill, 2007; Suárez-Domínguez *et al*., 2011). It is therefore imperative that measuring the impacts of non-consumptive anthropogenic activities on wildlife is made a priority in order to ensure effective management of these at risk areas (Beale, 2007).

While many researchers encourage the identification and protection of key habitats of conservation concern (Ikuta and Blumstein, 2003; Gill, 2007), it must be noted that well-managed visitation to observe even rare, and endangered wildlife can have a positive feedback for conservation, both socially and economically (Ellenberg *et al*., 2009). In addition to this, human access to wildlife areas is a key constituent in generating public support for the maintenance of spaces for biodiversity conservation (Gill, 2007). Managers cannot simply disregard the requests of tourists; since visitor satisfaction ensures continued economic returns to both local and national communities (Semeniuk *et al*., 2010). Nature based experiences can provide an important social and economic underpinning for wildlife conservation (Klaassen *et al*., 2006; Ellenberg *et al.,* 2009; Christiansen *et al*., 2010). This has led to a conflict of interests between local communities, who could potentially benefit from visitation by tourists, and the possible negative impacts that uncontrolled visitor access could have on the target species (Cassini *et al*., 2004). In addition to this, if tourist actions are left unbridled, they have the potential to diminish the visitor experience by deteriorating the quality of the natural environment to which they were first drawn (Semeniuk *et al*., 2010). Consequently, as wildlife tourism continues to increase in popularity, optimizing the relationship between the tourist experience and the focal species' needs has become a fundamental goal for conservationists (Semeniuk *et al*., 2010).

1.2 A discussion about natural and anthropogenic disturbances

The number of theoretical investigations into the allocation of resources in individuals has been extensive (Trivers and Willard, 1973; Maynard Smith, 1980; Ono *et al*., 1987). With the exception of Trivers and Willard, (1973), such theories have focused on the expected patterns of resource allocation under normal environmental conditions (i.e. those conditions experienced by an individual without any anthropogenic

disturbances present). However, in many locations around the world, populations or individuals are affected by natural and anthropogenic disturbances in their environment. Disturbances are a key component of many ecosystems. Disturbances have the potential to affect every dimension of a bionetwork, and can span both spatial and temporal dimensions (Fraterrigo and Rusak, 2008). As disturbances have origins which can either be natural or anthropogenic, disturbances are known to be inherently diverse (White and Jentsch, 2001; Fraterrigo and Rusak, 2008). Within specific ecosystems, disturbances might have non-uniform effects due to the fact that ecosystems are themselves heterogeneous in relation to their abiotic and biotic characteristics (Fraterrigo and Rusak, 2008). Disturbances are often defined as broadly any relatively distinct event in time that disrupts a community, population or individuals within a population (Suryan and Harvey, 1999).

All organisms encounter heterogeneity of some description. Even natural disturbance events can have significant impacts upon species and the surrounding ecosystem. Natural disturbances are perturbations in the environment that would occur even without the presence of humans. For example Ono *et al*., (1987) showed how the effects of natural disturbances can be felt over large time scales, and cause declines in pup survival in the California sea lion (*Zalophus californianus*) in the face of the 1982 El Niño event. During the El Niño time there is an increase in both sea level, and sea-surface temperature in the eastern Pacific Ocean (Cane, 1983), which is typically associated with an increase in salinity, and a decrease in the both the zooplankton and sea lions populations (Barber and Chavez, 1983; McGowan, 1984). During the 1982/83 El Niño event in the Pacific Ocean, changes in the oceanic conditions reduced the food stocks that the inhabiting pinniped populations relied upon; which led to a subsequent reduction in the birth size and early survival rate of offspring in several pinniped populations in Peru, Mexico, and California (Trillmich and Ono, 1991). Ono *et al*., (1987) revealed that both in the El Niño year, and the year after, pups spent less time suckling, were less active, and played less on land (Ono *et al*., 1987). Maternal investment, as measured by milk intake of offspring, was decreased and concurrently pups grew more slowly, and suffered higher rates of mortality during the El Niño year (Ono *et al*., 1989).

In addition to natural abiotic phenomena which impact offspring survival rate, a further natural disturbance source which has been shown to affect the breeding behaviour of colonially breeding mammals, is the density and sex-ratio of surrounding conspecifics. Boness *et al*., (1995) study on harbour seals (*Phoca vitulina*) revealed that females

tended to give birth in a seven day period at the start of the breeding season, when the operational sex ratio was in favour of females; with a ratio of 24 females per single male. Females which gave birth late in the breeding season, when males outnumbered females on the colony were disturbed by males three times more often than females that gave birth during the peak and as a result late pupping mothers spent 22% less time suckling and produced weaned pups that were 16% lighter than those females that pupped at the peak of season (Boness *et al*., 1995).

Individual species have evolved to cope with these natural disturbances as these are the selective pressures under which they have evolved. However, over recent years there has been a "new" pressure on wild fauna in the form of anthropogenic disturbances; often originating from sources such as ecotourism, and industrialisation of natural areas. The definition of anthropogenic disturbance used in this thesis is described by Nisbet, (2000), this being; "any human activity that alters the behaviour, and/or physiology of one or more individuals in a population''. So how do individuals respond to this "new" pressure of anthropogenic disturbance stimuli? Numerous studies have documented the effects that these more recent ecotourism activities have on wildlife behaviours such as: increased habituation to regular disturbance stimuli, a reduction in time spent in maintenance behaviours; hormonal changes; and decreased survivorship and/or reproductive success in an individual (Fowler, 1999; Lacy and Martins, 2003; Martin and Reale, 2008). In order to study the effects of anthropogenic disturbances it must first be established how individuals have been shown to respond to anthropogenic disturbances in prior studies. It is important to question whether the responses of populations and individuals to anthropogenic disturbances are similar to those exhibited in natural disturbance events, and whether similar responses to certain disturbance stimuli are maintained across species.

When discussing the effect that anthropogenic disturbances have on a population it is important to realise that an animal perceives disturbance stimuli that we might consider as low impact quite differently (Cassini *et al*., 2004; Stankowich, 2008; Ellenberg *et al.,* 2013). For example in birds, prolonged motionless observation for determination of nest status is generally regarded as being less stressful than a short direct approach to the nest; but in Yellow-eyed penguins (*Megadyptes antipodes*) it has exactly the opposite effect (Ellenberg *et al.,* 2013). Ellenberg *et al.,* (2013) found that the duration of a stimulus was the key factor in determining the level of response by an individual, as indicated by an elevated heart rate until a person fell out of sight. Ellenberg *et al*., (2013) determined that human activity was the next most important factor; with a

moving wildlife photographer eliciting a higher heart rate response than an entirely motionless human at the same distance from the individual (Ellenberg *et al.,* 2013).

Over the past two decades, there has been increasing research into the effect that vehicular machines have on species in their natural environment; with particular emphasis being placed on the effects that boat and aerial disturbances have on individuals. The Donna Nook site where this current study took place is subjected to levels of aircraft disturbances from the adjacent Ministry of Defence site. Prior studies related to the responses of individuals to aircraft disturbances typically range from slightly increased vigilance behaviour to a flight response, where animals flee from the affected area, either on a short term or in extreme cases, a long term basis (Bleich *et al*., 1990; Tracey and Fleming, 2007). A study on bighorn sheep (*O. canadensis*) in the Grand Canyon revealed that responses to overhead disturbance sources have the potential to alter the time budget of species. In this study, helicopter fly overs reduced the amount of time in which bighorn sheep spent foraging by 17% (Stockwell and Bateman, 1987; Stockwell *et al*., 1991; Tracey and Fleming, 2007).

Previous studies have suggested that helicopters cause a more intense alert response than fixed-wing aircraft in a number of megafauna species (Grubb and King, 1991; Harrington and Veitch, 1991), however an individual's behavioural responses to different models of helicopter or fixed wing aircraft have rarely been compared. Tracey and Fleming's', (2007) study on goats discovered that individuals did not exhibit a uniform response to aircraft disturbances. The height and model of the aircraft altered the response exhibited by individuals; with individuals showing a heightened alert response to the larger and louder Hughes 500 helicopter when compared to individual responses to the smaller Bell Jetranger helicopter (Tracey, 2004). This difference in response to the two helicopters by the feral goats suggests that the goats' responses to the helicopter are to the type of sound as well as noise level, with the Hughes 500 helicopter being more audible from the ground that the Bell Jetranger helicopter (Tracey and Fleming, 2007). Born ***et al.'s*** (1999) study on seals in the Dollard estuary supports the finding by Tracey and Flemming (2007). Born *et al.* (1999) found the escape response of ringed seals (*Phoca hispida*) to be related to the type of aircraft, with a stronger response to helicopters than to fixed-wing aircraft (Osinga *et al.,* 2012). In addition to this, Born *et al.,* (1999) found that when aerial disturbances do occur they appear to disturb the seals by the sound emitted rather than by the visual cues of the vehicles.

The responses of marine mammals to aircraft noise are complex and sometimes poorly understood (Richardson et al. 1995). Responses may depend on factors such as hearing sensitivity, habituation, and the presence of offspring. Behavioural responses of populations could range from subtle changes to resting and foraging patterns to active avoidance or escape from the region of disturbance. Age and sex are important factors in noise sensitivity due to aircraft. For instance, juvenile and pregnant Steller sea lions (*Eumetopias jubatus*) are more likely to leave a haul-out site in response to aircraft disturbances than females with young (Calkins 1979; Hildebrand, 2005).

Boat disturbances primarily affect species which are found in aquatic and coastal environments. Tripovich *et al,* (2012) looked into the effect that motor boat noise had on Australian fur seals (*Arctocephalus pusillus*). The experiment examined and revealed that fur seals use vocal plasticity to cope with alterations in anthropogenic noises such as changes in the amplitude of the boat noise. The results suggest that these seals perceived the boats as potential threats; with louder motor boat noise, initiating a greater aggressive and alert behavioral response (Tripovich *et al*., 2012). The study assumed that the response to these extreme sound levels generated energetically costly behaviours involving the seals either orientating themselves towards the boat noise, or physically moving away from the noise source (Tripovich *et al*., 2012). Similar results were seen for other pinniped colonies and other marine mammals (Cassini *et al*. 2004).

On land, there are numerous aspects of a pedestrian's approach which may affect the strength of a response by a population, these include: the approach distance; visitor group size and behaviour; and the predictability of the disturbance source (Antarctic Treaty consultative Meeting, 2008). Susceptibility to a source of anthropogenic disturbance can be both species-specific, and within a population, individual specific. Responses to anthropogenic disturbance might be in addition to this, influenced by parameters such as the presence of, or distance to, a shelter, the location of any offspring, and the density and composition of the population in which the individual is located. In general, hunted populations have been revealed to show significantly greater flight responses than non-hunted populations (Stankowich, 2008). In addition to this, in areas where hiking is common, humans which hiked in a predictable hiking context (i.e., on trails) were less threatening than humans hiking off trails (Stankowich, 2008).

A major factor influencing these decisions in encounters with people is the person's distance to an individual within the population (Cassini, 2001), and the mode of their approach to the animal. A study on New Zealand fur seals (*Arctocephalus forsteri*), found that when tourists approached a distance of less than 5 m individuals reacted with threatening behaviour (Pavez *et al.,* 2011). Having said this, a study by Renouf *et al.,* (1981) found that harbour seals exhibited a very high tolerance to approaching pedestrians during the breeding season. In addition, seals were observed to return very promptly after, and even before, the departure of pedestrians during the breeding season emphasizing the seals' strong association with land during the breeding season (Cunningham *et al*., 2009; Andersen *et al.,* 2011).

A study on elk (*Cervus elaphus*) by Ciuti *et al*., (2012) indicated that it was not just the number or distance of people, but above all it was the behaviour and composition of the tourist group which moulded elk behaviour. The study found that higher numbers of tourists can have an overall reduced effect on elk if the type of human activity is relatively benign, i.e., the effect of hikers on elk behaviour was lower than that of motorised recreational activities (Ciuti *et al*., 2012). In South American fur seals, tourists shouting and running elicited a more negative response than those walking and speaking in low voices (Cassini, 2001; Cassini *et al*., 2004). Cassini *et al*., (2004), used voice level as one factor in rating the intrusiveness of tourists. In the study Cassini *et al., (*2004) observed a much higher percentage of flush response and aggressive seal behaviour, when the tourists behaviour was classified as intermediately to intensely disturbing rather than calm (Cassini *et al*., 2004).

Few published studies (Burger and Gochfeld, 2007) have quantitatively considered the effect of visitor group size on wildlife; although many studies have investigated the effect that approach distance has on wildlife. This is surprising since it has been demonstrated that if the visitor numbers in an area fluctuates spatially and temporally, then fixed barriers are unlikely to be effective (Beale and Monaghan, 2004; Antarctic Treaty consultative Meeting, 2008). Cassini *et al*., (2004) study on South American fur seals noted fa visitor group size effect on individuals within the effected population: families increased the mean number of fur seals reacting per approach. This result may have been affected by the behaviour of tourists as well, since the chances of at least one member of the group showing intrusive behaviours increasing with the size of the group (Cassini *et al*., 2004).

1.2.1 Sensory cues

The introduction has so far primarily discussed the effects that visual disturbances such as aircraft and pedestrians have on the surrounding fauna; however it must be borne in mind that disturbances are triggered by all senses, and it is not only visual cues which have the potential to impact upon an individual's behaviour; other sensory cues can also have significant impacts on an individual's behavioural repertoire such as auditory and chemical cues. For example, studies on marine mammals have suggested that anthropogenic noise can have many effects on both their behaviour and physiology. Auditory stimuli can bring about changes in vocal behaviour, such as alterations in an individual's call duration or repetition rate, and changes to the frequency or amplitude of call components (Antarctic Consultative Treaty, 2008). In extreme cases, marine mammals may stop calling altogether (Foote *et al*., 2004). Furthermore auditory disturbance events can lead to changes in movement patterns in order to avoid the auditory cue altogether (Henry and Hammill, 2001). In particularly severe cases, auditory disturbances can lead to physical injury or death of an individual (Richardson *et al*., 1995; National Research Council, 2003). For anticipatory management decisions, it is important to determine the relative severity of different auditory stimuli on stress level (Ellenberg *et al*., 2013).The fact that stress in animals is induced by human disturbance on animals is now widely accepted (Carney and Sydeman, 1999; Dyck and Baydack, 2004; Martin and Reale, 2008), yet few studies have investigated how different auditory stimuli affect the way that individuals behave in their natural environment (MacDougall *et al*., 2013).

With regard to the impact of scent; olfactory cues have been shown in a number of studies to evoke or alter the behavioural response of individuals in a range of taxa. For example, field estimates of seed removal rates are often determined by monitoring the survival of seeds placed at stations. Such experiments may unintentionally provide seed predators, such as rodents and insects with unnatural olfactory cues (Duncan *et al*., 2002). Duncan *et al.,* (2002) compared the removal of seeds that had direct contact with human skin with those seeds which had no contact with human skin. Rodents are seed predators in many systems and have an acute sense of smell (Vander Wall, 1995, 1998). If researchers leave unnatural olfactory cues such as their own human scent on the seeds, rodents may detect these experimental seeds more than the naturally dispersed seeds. This could lead to differences in the seed dispersal patterns of a habitat which is perturbed which could ultimately affect the amount and density of

resources in different parts of the habitat exposed to different levels of human disturbance. The study by Duncan *et al.*, (2002) found that seed removal was greater for seeds touched by researchers than those that were unscented. The effect of scent on the removal of the seeds was pronounced during the first week, and then disappeared, suggesting that the scent biases were weak and short-lived (Duncan *et al.,* 2002). Furthermore, studies on the impact that scent left by anthropogenic sources has on the life history of species, have revealed that unnatural scents may impact upon the survival rate of offspring. For instance, studies of avian nest predation which used artificial nests and/or artificial eggs to quantify egg predation found that there were higher predation rates on artificial rather than natural nests (Ortega *et al.,* 1998, Sloan *et al.,* 1998; Wilson *et al.,* 1998; Skagen *et al.,* 1999; Zanette and Jenkins, 2000; Duncan *et al.,* 2002). Similarly, Whelan *et al.,* (1994) found predation at artificial nests monitored by researchers wearing commercial deer scent, which mask unnatural human odours such as perfumes, body lotions and natural human scents, was lower than predation at nests visited by researchers wearing perfume, or where no scent manipulation took place. The results of these studies suggest that scent is a possible factor that we must consider when looking at the impacts that disturbances have on the behaviour of species in their natural environment when they are subjected to high levels of ecotourism.

1.3 The generalised impacts of disturbance on animal taxa

Anthropogenic disturbance in natural environments is a significant catalyst of habitat change, with potentially important implications for individuals, populations and communities (Gill, 2007; Anderson *et al.,* 2011). Anthropogenic disturbances have the potential to influence many components of a species' behaviour and physiology (Ciuti *et al*., 2012). Changes in the behaviour of an individual or population as a result of a disturbance stimuli may either occur directly, or indirectly by influencing aspects of an individual's being that determine fitness, and which may prompt a behavioural response (for example, a reduced prey availability) (Fortin and Andruskiew, 2003; Tuomainen and Candolin, 2010; Andersen *et al.,* 2011). For instance, human disturbances have been reported to negatively impact upon the breeding success of penguins (Ellenberg *et al*., 2009), while in brown bears (*Ursus arctos*) the occurrence of human disturbance was found to increase the brown bears energetic expenditure as a result of behavioural modifications in the species (Ellenberg *et al*., 2009; Tripovich *et al*., 2012). Further to this point, any initial behavioural modifications in response to a disturbance event elicited by a population may over time proceed to influence the

reproductive success and distribution of the effected population; which has the potential to ultimately influence the biodiversity of the ecosystem (Tuomainen and Candolin, 2011; Andersen *et al.,* 2011; Benoist *et al*., 2013).

1.3.1. Mitigation Measures

As the number of tourists wanting wildlife encounters continues to grow, management strategies are needed in order to limit the impact on those species subjected to anthropogenic disturbances. One approach is to restrict public access with a physical barrier, under the assumption that a barrier will provide a refuge for the wildlife (Ikuta and Blumstein, 2003). Other forms of barriers include posting signs (Erwin, 1989; Nordstrom *et al*., 2000); but these may only be effective in places rarely visited by tourists. In sites that experience high levels of tourism, physical barriers such as fences, may be the most effective way to prevent direct human interactions with wildlife (Burger *et al*., 1995; Ikuta and Blumstein, 2003). Ikuta and Blumstein (2003) found that birds located in highly visited areas which are protected by a fence line responded similarly to birds located in areas that are subjected to low levels of visitation, and behaved significantly differently from those birds located at sites with no fences that are prone to high levels of visitation by tourists (Ikuta and Blumstein, 2003). This study suggests that by reducing the number of tourists at sites, and providing areas of refuge for focal species, protective barriers allow individuals to behave as they would in an undisturbed environment (Ikuta and Blumstein, 2003). This finding was consistent with other studies which found that habituation was more likely to occur with repeated exposure to humans when a barrier was in place (Cooke, 1980; Lord *et al*., 2001).

1.3.2 Habituation and sensitisation to disturbance events

The behavioural response of an animal to a specific disturbance event is likely to be correlated to, as described in section 1.2., multiple interacting factors; for example tourist group size and behaviour (Cassini *et al*., 2004; Baird *et al*., 2005), and the type of disturbance involved (Rodgers and Smith, 1997; Rees *et al*., 2005). In addition to this, the cumulative effect of repeated exposures to the disturbance will affect the behavioural response of the effected population, leading to either sensitization or habituation (Bejder *et al*., 2009; Andersen *et al*., 2011). A population level response to a disturbance event is defined in this study as the long term effects on the general activity budgets of a population in response to a disturbance event and any long term changes to the survival rates and demography of a population as a result of these

disturbance events (Suryan and Harvey, 1999; Stankowich, 2008). Here, habituation is defined as a diminishing of a response to a frequently repeated stimulus (Krausman *et al*., 2004). Contrastingly, sensitization occurs when repeated administrations of a stimulus results in the progressive amplification of a response. Sensitization often leads to an enhancement of behavioural and physiological response to a whole class of stimuli in addition to the one that is repeated.

There is an argument that regular exposures to benign human activity can be tolerated and accepted by an individual (Van Polanen Petel *et al*., 2008; Jiang *et al*., 2013). Regular, non-threatening disturbances may enable animals to become habituated to the disturbance source, and thus reduce the intensity of their reaction to the distance (Jiang *et al*., 2013). The degree of habituation may vary amongst individuals within a population (Picton, 1999; Stankowich, 2008); and individual variation in habituation potential may be dependent on previous experience with humans (Ellenberg *et al*., 2009). For example, ungulates in areas with frequent contact with humans showed reduced flight responses compared to those ungulates in areas where human contact is rare. This result is in agreement with studies of other taxa (Blumstein *et al*., 2003; Cooper *et al*., 2003). Signs of habituation include a reduction in a behavioural response to a stimuli or increase in reproductive success. The repeatability of this effect across studies suggests that some species do habituate to humans in heavily populated areas (Stankowich, 2008). One explanation for this lack of response may be due to the motivation to stay or leave an area by an individual based upon the perceived quantity, or quality of resources in that patch of habitat. Where there is a high quantity or high quality of resources in an area there may be a correspondingly high motivation to stay in the area regardless of the sources of anthropogenic disturbance, thus explaining the observed apparent habituation by individuals (Gill *et al*., 2001).

Support of the idea that populations can habituate to human presence can be found in a study of Weddell seals (*Leptonychotes weddellii*) by Van Polanen Petel *et al*., (2008) which investigated the effect of repeated pedestrian approaches over a short-time period (two hours) had on the behaviour of lactating seals. The study revealed that seals exhibited evidence of rapid habituation to human presence; with a reduction in the proportion of seals that responded to the disturbance source; with 67% looking up during the first approach compared to 18% during the tenth approach by pedestrians (Van Polanen Petel *et al*., 2008).

Van Polanen Petel *et al*., (2008) study also revealed the importance of studying populations over a long time period in order to gain a complete picture of a population's response to a disturbance source. The study, in addition to looking at the immediate effects of disturbance, looked at the effect of irregular pedestrian activity over a long-time period (approximately 3 weeks) on seal behaviour. Analysing the results over a longer time frame revealed that seals did not habituate to the disturbance source over this longer time period; rather adult female seals became more sensitised to pedestrian approaches (Van Polanen Petel *et al*., 2008). Mellish *et al.,* (2010) supported the findings of Van Polanen Petel *et al*., (2008) study, reporting that varied levels of pedestrian traffic within a two-hour window resulted in habituation of Weddell seals, but repeated exposure over a longer period had the opposing effect and resulted in sensitization to the disturbance source (Van Polanen Petel *et al*., 2006; Mellish *et al*., 2010). These studies suggest that there is a potential for increased stress among individuals when exposed to repeated but irregular disturbances over a single breeding season which have the potential to affect the reproductive rates of females on the colony and the survival of pups (Mellish *et al*., 2010).

In addition to the numerous studies which have chiefly focused on habituation to visual stimuli, there is a wealth of studies which have alluded to the fact that some populations have seemingly habituated to auditory disturbance stimuli present in their environment. Temporal fluctuations in the reactions of wildlife to auditory stimuli are well documented (Koehler *et al*., 1990), with a reduction in behavioural responses to anthropogenic disturbance stimuli often noted during the breeding season. Most animals are able to habituate to sounds discharged at regular intervals, and where the possible threat to the individuals is not reinforced (Thompson and Spencer, 1966; Tracey and Fleming, 2007). For example, Muskoxen (*Ovibos moscharus*) habituate to regular helicopter flyovers (Miller and Gunn, 1980) and a population of pronghorn (*Antilocapra americana sonoriensis*) which inhabit an air force base have been shown to have habituated to the noise produced from overhead military flyovers (Krausman *et al*., 2004). Krausman *et al.,* (2004) study on pronghorn indicated that individuals exposed to regular military flyovers behaved similarly to individuals in locations where military flyovers were absent. Behavioural activity budgets of the pronghorn located near to the military site were found to be similar to pronghorn which were not exposed to military flyovers. From this result Krausman *et al.,* (2004) concluded that the pronghorn sheep near to the military site had habituated to the noise generated from the flyovers over the herd. At the Donna Nook site where this current study is conducted, the seals are subjected to regular flyovers by RAF military aircraft and it

would be interesting to note whether the seals at the Donna Nook colony show signs of habituation to not only the visual disturbances at the site but also the auditory stimuli centred around the military flyovers.

It is recognised that the absence of notable behavioural responses does not necessarily indicate habituation (Beale and Monaghan, 2004). Increasingly physiological evidence of habituation is being sought to confirm whether habituation has indeed occurred within a population (Antarctic Treaty consultative Meeting, 2008). Even in cases where animals at a site show apparent evidence of habituation, it should be borne in mind that the disturbance may have merely caused the less tolerant individuals to abandon the site, leaving behind only the most tolerant individuals (Antarctic Treaty consultative Meeting, 2008). Wrongful application of the term habituation can mislead wildlife managers to conclude that anthropogenic activity has benign consequences for wildlife which has the potential to seriously undermine management plans for an area (Bejder *et al*., 2006).

1.4 How responses to disturbances are measured

There are numerous ways in which it is possible to study and measure an animal's response to disturbance events, but changes in an individual's behavioural repertoire is often the first, and also the most obvious consequence of anthropogenic activities; so it is not surprising that given this, many researchers use behavioural observations to gather evidence for the possible effects that disturbances have on individuals (Fortin and Andruskiew, 2003; Nettleship, 1972; Beale, 2007; Benoist *et al*., 2013). Behavioural responses have the added benefit of being able to provide fast evaluations of how individuals are reacting to sources of disturbance in their natural environment. For instance, it has been proposed that certain behavioural responses, such as vigilance levels, can be used to estimate an individual's tolerance to a particular disturbance event, which can then be used to guide management actions at local, regional and possibly even national scale in order to reduce these behavioural impacts (Lima and Dill, 1990; Fox and Madsen, 1997; Gill *et al.,* 2001; Stankowich and Coss, 2007). Tolerance is here defined as the capacity of an individual to endure subjection to a disturbance event without an adverse reaction.

In order for scientists to study the effects that anthropogenic disturbances have on a population, scientists must first be able to note the general patterns of behaviour of individuals within a population under normal conditions (i.e. with no human

disturbances) throughout different life stages of the species. It is only then that scientists can start to discern what behavioural responses may be linked to a disturbance event and what behaviours may just be part of their everyday repertoire. After an indication of the types of responses to disturbance event by an individual have been identified, defining the possible behavioural responses of anthropogenic disturbance which have the highest biological significance in terms of threatening the survival and successful reproduction of the population is critical for maintaining healthy populations of the species (Carney and Sydeman, 1999; Gill *et al*., 2001; Engelhard *et al*., 2002).

Disturbed animals will often undertake vigilance behaviours to evaluate the potential danger to themselves and possibly their offspring and kin (Dyck and Baydack, 2003; Cassirer *et al*., 1992). In this respect, it is possible that measuring the vigilance responses of individuals in disturbed populations could be a useful way in which to measure the effects of disturbance events on targeted populations. Vigilance patterns are often moulded by: the density/ proximity of predators; human disturbance patterns; and the population's abiotic habitat. For instance, upon encountering pedestrians, a number of bird species have been shown to increase the time they devote to vigilance behaviours, and diminish their rates of foraging (Fernández-Juricic and Tellería, 2000). In extreme cases they may even flee the disturbed site altogether (Miller *et al*., 1998; Fernández-Juricic and Tellería, 2000). The principal cost of vigilance is thought to be time, where opportunities for alternative behaviours are lost, with the most common of these trade-offs occurring between vigilance and foraging behaviours. Many theoretical models assume that vigilance is irreconcilable with foraging behaviours and many studies have indeed recorded that time spent vigilant is usually inversely correlated with time spent feeding (Cassini *et al*. 2004; Gill, 2007; Ciuti *et al*. 2012). For example, a study by Roe *et al*., (1997) showed that the presence of humans triggered an increase in vigilance and decrease in foraging behaviours in elk (*Cervus Canadensis*) (Roe *et al*. 1997; Wolf and Croft, 2010). In addition to such trade-offs between vigilance and maintenance behaviours (these being behaviours associated with comfort movements, exploration and foraging), vigilance levels of many species have been shown to increase in females with offspring, which may impact on the proportion of time a female can afford to spend nursing her offspring, which may ultimately impact on the survival probability of the affected offspring (Wolf and Croft, 2010).

1.5 Species and individual response variations to disturbance events

Prior research on species' responses to anthropogenic disturbance has supported the idea that susceptibility to various sources of anthropogenic disturbance is likely to be species-specific (Antarctic Treaty consultative Meeting, 2008). However, behavioural responses to disturbances are always context-dependent and individual responses to human presence will therefore depend on the trade-offs experienced by those individuals within a population; meaning that not only are responses to disturbance species specific, but often they are also individual specific (Gill, 2007). For example, the decision to stay or to leave an area by an individual in response to a disturbance event will ultimately be influenced by: the quality of the area in terms of its resources; the availability and relative quality of alternative areas which the individual could move to; and the disturbance source (Gill, 2007). In addition to this, there are number of other intrinsic and contextual factors which have been shown to impact upon an individual's tolerance level to a disturbance event including: colony size and composition; time of day or year; stage of breeding; and variations in an individual's age, size, condition, and personality. For example, conclusions based on Clemmons *et al*., (1997) study suggest that during the breeding season, the occurrence of stressful events may redirect an individual's behaviour towards survival rather than reproduction; and consequently, increase the possibility of offspring abandonment (Clemmons *et al*., 1997, Ellenberg *et al*., 2013). Due to the diversity of factors which have the potential to impact upon the type and level of disturbance response exhibited by individuals; it is no wonder that much individual variation, even within a population exists in reaction to a particular disturbance event (Antarctic Treaty consultative Meeting, 2008). The following two sections will discuss in greater detail the effects that breeding context and intrinsic factors have on an individual's response to a disturbance event.

1.5.1 Breeding Context

From a conservation perspective, human disturbance of wildlife in the past has been considered only important if it is known to affect the survival and/or fecundity of the species, and hence cause the population to decline. It was therefore vital for conservationists to know whether the effects of disturbance stimuli do result in a decline in the size of a population (Gill and Sutherland, 2000; Gill *et al*., 2001). This prior conservational perspective has over recent decades been overturned and there are now numerous projects which look to conserve environments that are not yet

thought to be under threat of extinction but are exposed to anthropogenic disturbances (Foster et al., 2003; Bassett et al., 2004). Anthropogenic disturbances can be particularly detrimental during certain critical periods of an animal's life when animals are in a vulnerable condition such as during pregnancy and nursing (Phillips and Alldrege, 2000). In female mammals lactation is a period of maximum energetic drain and therefore may present a time when animals may be most vulnerable to a disturbance event. If this is indeed the situation, the reproductive success of the population would likely be affected (Bejder *et al*., 2006). Within colonies, females may be more sensitive to disturbances at certain stages of breeding season, since parental defence of offspring is likely to increase as the breeding season progresses due to the high density of individuals found at breeding colonies, and the higher male: female operational sex ratios found later in the breeding season (Newby, 1973; Côté, 2000; Antarctic Treaty consultative Meeting, 2008). Within a breeding season, disturbance stimuli may have the ability to change the location of breeding sites, by discouraging first-time breeders from settling near sources of disturbance (Antarctic Treaty consultative Meeting, 2008). Consequently the density of females at breeding sites may be affected at disturbed sites which have the potential to affect the reproductive success of individuals at these disturbed locations.

When evaluating the effects that disturbances have on individuals during the breeding season, it is important to note that both the type and intensity of the behavioural / physiological response may vary depending on the stage of the breeding season. For example, human disturbances which occur early in the breeding season in penguins have been known to cause not only egg loss but also nest abandonment (Hockey and Hallinan, 1981). Having said this, once nests are established, most penguin species show negligible behavioural responses to human disturbance (Nimon *et al*., 1995), which can often be mistaken for habituation (Seddon and Ellenberg, 2008; Ellenberg *et al*., 2009; Ellenberg *et al*., 2013). This change in behavioural response is not uniform across all taxa and even varies between penguin species. For instance, Wilson *et al*., (1991) noted, in contrast to Ellenberg *et al*., (2013) that Adélie penguins (*Pygoscelis adeliae*) attending chicks late in the breeding season will often flee from the area if approached by pedestrians to a distance within 6 m. This is in contrast to the behaviour of the females with young chicks, where adults will tolerate pedestrian approaches within 1 m of the nest site (Wilson *et al*., 1991).

For long-lived females breeding experience and current environmental conditions can vary widely and have been known to impact upon offspring survival (Hadley *et al*.,

2007).Many studies have investigated how various parental traits impact offspring size, development, and survival (Dejesus and Hirano, 1992; McCormick, 1998). There is general agreement that fecundity and offspring size and survival at independence are related to a female's age and body size (Bernardo, 1996). There is some consensus that disturbance events may alter the condition of the females of a species at parturition which may in turn impact upon the survival chances of the offspring they produce (Antarctic Treaty consultative meeting, 2008). Variation in a female's response to a disturbance event during the breeding season is generally accounted for by a combination of maternal characteristics such as age and previous experiences with a disturbance source.

1.5.2 Intrinsic differences between individuals within a population

In addition to the type of disturbance, and whether the disturbance occurs within or outside the breeding season, the tolerance of an animal to human proximity varies with the species, time of day, and other life history traits such as age, size, condition, current behavioural state, and previous experiences with a particular disturbance stimuli (Gill *et al*., 2001; Ellenberg *et al*., 2013). For example, in yellow bellied marmots, juveniles spent significantly less time vigilant than yearlings and adults when faced with anthropogenic disturbances (Li *et al*., 2011).

With respect to sex, the results of some studies seem to indicate that males and females appear to be affected differently by human activity (Childress and Lung 2003, Ciuti *et al*., 2004; Lykkja *et al*., 2009), possibly as a results of the variations in life histories between the two sexes. For instance males of many species have to compete to win mates whereas most females that are of breeding age will be mated within a breeding season; however after birth females of many species will solely look after offspring with little to no paternal engagement. In addition to this females often have a longer reproductive lifespan than males, and often reach maturation at an earlier age. These differences in life histories may make the different sexes more responsive to disturbances at different stages in their life cycle. For example, it might be expected that males might be more risk-taking than females due to the fact, as mentioned previously, males of many species such as many pinniped species do not have to protect their offspring after birth (Lykkja *et al*., 2009). Barton *et al*., (1998) found a differential response between male and female New Zealand sea lions (*Phocarctos hookeri*) to tourist presence; females tended to respond negatively to disturbance events and exhibited a more intense response when visitors approached at a shorter

distance. In contrast, males defended their territories from disturbances, and responded with more aggressive behaviours directed at tourists at greater distances, and continued with aggressive bouts for a much longer time than females (Barton *et al*., 1998; Pavez *et al*., 2011).

Many studies have investigated how various parental traits impact offspring size, development, and survival (Dejesus and Hirano, 1992; McCormick, 1998). In most mammalian species, females bear all the direct costs associated with producing offspring; which include those for gestation, birth, lactation and parental care (Anderson *et al*., 2011). In addition to this, for long-lived iteroparous females, the success of any one reproductive season may be influenced by their efforts in their preceding breeding seasons (Newton, 1989; Pomeroy *et al*., 1999). Variations in an individual's response to a disturbance event during the breeding season may also be dependent upon the sex of the female's offspring (Smiseth and Lorensten, 1995b). In polygynous, sexually dimorphic species, parents may be predicted to bias their parental investment towards sons, and so spend a higher proportion of their time engaging in nursing and protective behaviours, and staying within a closer proximity with a male offspring than a female young. This is due to the fact that adult males commonly experience variations in reproductive success whereas most reproductively active females at a breeding colony will be mated by a male and so will have a fairly uniform reproductive success rate (Trivers and Willard, 1973). Moreover, male reproductive success is often dependent upon their adult body size, which, in turn may depend on the level of parental investment they received as an infant (Smiseth and Lorensten, 1995b). No study, as of when this thesis was published has looked at whether this bias between the sexes remains under disturbed conditions.

Offspring fitness is derived partly from the input of the mother, in terms of the amount of parental care and provisioning derived from their mother (Evans, 1990). In the evolutionary concept of life history theory, natural selection is supposed to not only optimise the chances of offspring survival, but also optimise parental fitness (Stearns, 1976; Georges and Guinet, 2000). Females may vary in condition not only from one another but the same individual may vary in condition from one breeding season to the next (Pomeroy *et al*., 1999). In this respect individual females may vary their responses to disturbance events during the breeding season based on their body condition and age. Environmental changes and disturbance events may influence the rates of parental expenditure on offspring by altering the accessibility of resources to individuals (Ono *et al.,* 1987; Pomeroy *et al.,* 1999). When disturbances elicit extreme responses

from individuals, females may exert more of their internal resources into their own survival resulting in a correlated reduction in parental investment into the young produced in that year (Pugesek and Diem, 1983; Evans, 1990). During reproductive events, parental strategies are expected to optimise the rate of energy acquisition of the offspring (Coulson, 1968; Pomeroy *et al*., 1999; Georges and Guinet, 2000).The theory of state-dependent life history evolution predicts that mothers of different physiologic states might have different maternal care tactics, with females in better condition investing more in her offspring than those in poor body condition (Chastel *et al*., 1995; McNamara and Houston, 1996; Georges and Guinet, 2000). During periods of anthropogenic disturbance during the breeding season, those females which show a heightened response when compared to the rest of the population may favour investment into their own survival, thereby reducing their investment in their own pup, potentially affecting the pup's long term chances of survival. An individual's response to a disturbance event may depend on an individual's age and prior experience to a disturbance, thus suggesting that an individual's response to a disturbance event may alter the partitioning of resources between mother and pup by redirecting resources to their own survival rather than the survival of their pup (Georges and Guinet, 2000).

In a wide range of species, individual differences in behavioural reactions when facing challenges such as disturbance events remain consistent over time and across situations (Réale *et al*., 2007; Fernández-Juricic, 2000; Twiss *et al.,* 2012). These behavioural consistencies often referred to as temperaments, or personalities have chiefly focussed on non-reproductive contexts. Nevertheless, many vertebrates can present individual differences in relation to reproductive behaviours, commonly termed mothering styles (Twiss *et al.,* 2012). Mothering styles are defined as "the occurrence of consistency over a number of periods of maternal care with regard to relative differences between mothers for parameters of maternal behaviour" (Albers *et al*., 1999). In short, they are consistent individual differences (CIDs) in maternal behaviours, within a population, across a number of rearing periods. Mothering styles may affect how individual females respond to disturbances during the breeding season (Hill *et al*., 2007). Mothering styles have been identified in a range of non-human mammals, from rodents (Albers *et al*.,1999) to rhesus and Japanese macaques (*Macaca mulatta*, *Macaca fuscata*, respectively, Weaver and de Waal, 2002; Maestripieri *et al*., 2009), grey seals (*Halichoerus grypus*, Twiss *et al*., 2012) and humans (*Homo sapiens*, L., Meaney, 2001).

The proactive- reactive axis of personality may be a model which can aid in the explanation as to why there is a variation in response to disturbance events by individuals within a population. According to Koolhaas *et al*., (1999), reactive animals show higher cortisol release in response to a stressor than do proactive individuals (Martin and Reale, 2008). In general, proactive individuals tend to form routines, are more aggressive, and express limited flexibility in their behavioural repertoires compared to reactive individuals, who mold their behavioural response to an individual situation, and are more responsive to environmental stimuli such as disturbances (Twiss *et al*., 2012). It is known that the temperament of an individual (i.e. whether it is proactive or reactive), affects its dispersal (Fraser *et al*., 2001; Dingemanse *et al*., 2003); meaning individuals within a population may vary in their potential to occupy habitats with different amounts of anthropogenic disturbance in accordance with their temperament (Martin and Reale, 2008). As a consequence, endocrinal differences between animals occupying disturbed and undisturbed areas may not be solely a direct effect of stress response to disturbance by humans, but may also reflect the non-random spatial distribution of individuals of different temperaments (Martin and Reale, 2008). Martin and Reale's, (2008) results pointed out an important issue: individuals are distributed non-randomly according to their temperament across a disturbance gradient.

Despite the establishment of theoretical models (Weaver and de Waal, 2002) which have been developed in order to explain mothering styles, and the responses to disturbance in many species , there remains a lack of empirical evidence to support these models for selective mechanisms that maintain this variation in wild populations (McDonald *et al*., 2012; Twiss *et al*., 2012). A study by Twiss *et al*., (2012) examined whether behavioural types were present in a wild population of female grey seals at the North Rona breeding colony and then related this to fitness measures. The degree of change in pup-checking rates in the study by Twiss *et al*., (2012) across situations suggests a range of behavioural types, indicative of a proactive-reactive axis. Twiss *et al.,* (2012) study indicated that proactive females tended to perform pup-checking behaviours at a constant rate irrespective of the situation, indicating a very limited plasticity. In contrast to this, reactive females altered their pup-checking rates markedly between the undisturbed and disturbed conditions, displaying a high degree of behavioural plasticity in order to react to the environmental stimuli (Twiss *et al*., 2012).

Further to the comparison of individual behaviours across disturbance situations, this current study will also use a measure of mother-pup relationship quality adapted from

Weaver and de Waal, (2002) in order to test whether the quality of mother-offspring relationships in grey seals remained constant across disturbance contexts. The Mother-Offspring Relationship Quality (MORQ) index was originally used by Weaver and de Waal, (2002), to describe the quality of mother-offspring relationship in brown capuchin monkeys. This index was calculated based on a ratio of affiliative to rejective behaviour seen between mothers and their offspring, relative to the ratio of all other mother-young pairs within the study. This index allows identification of mother-offspring pairs with a more affiliative relationship and those with a more rejective relationship relative to the population as a whole.

1.6 Why is it important to study the effects of disturbance in marine mammals?

Although growth of the ecotourism sector has occurred in almost all natural areas, none have seen the surge in popularity with the general public greater than marine and coastal environments (Bejder *et al*., 2006; Garrod and Wilson, 2004); where the attractions of viewing large mammals such as cetaceans and pinnipeds with some predictability in the wild seems to have the 'wow' factor to attract large numbers of people, often willing to pay substantial sums of money (Hoyt, 2001; Strong and Morris, 2010). For example, cetacean watching, which targets at least 56 (including endangered and threatened) species, involves more than 9 million people a year and is worth approximately US$1 billion (Samuels *et al*. 2003; Bejder *et al.* 2006). Cetacean-watching tourism is commonly presented as a benign alternative to whaling (Hoyt, 1993), that enhances public attitudes toward the marine environment (Orams, 1997) and helps support local economies (Hoyt, 2001). Nevertheless, given the nature of this type of tourism, which often demands close encounters with the cetaceans, along with the that fact that specific cetacean communities are quite often small; there exists a considerable potential for harmful consequences in targeted animals (Bejder *et al.*,2006). It is therefore crucial that a greater assessment of the impacts of these disturbance events in marine mammals is built up to grasp a more complete understanding of their behavioural and physiological responses to human activity (e.g., Bejder *et al.,* 1999; Constantine *et al.,* 2004; Corkeron, 2004; Samuels and Bejder, 2004; Bejder *et al.,* 2006). While the number of people wanting to participate in ecotourism activities in aquatic and coastal environments continues to rise, wild aquatic and semi-aquatic mammals have received far less attention in terms of research into the impacts of disturbance than land mammals (Hoyt, 2001; Bejder *et al.,* 2006). This is presumably due to the fact that behaviour is far more difficult to follow and assess once

the focal individual enters an aquatic environment. As the unabated growth of nature-based tourism is forecast to continue at least for the foreseeable future, particularly in marine and coastal habitats, it is imperative, not only for ecological, but also for economic sustainability, to study the effects that anthropogenic disturbances have on the behaviour of affected populations in order to minimize the associated possible survival and reproductive impacts on the focal species (Orams, 1995; Ellenberg *et al*., 2013).

A major impediment to evaluating the biological impacts of noise on marine mammals is the gaps in our knowledge regarding marine mammal responses to sound (Hildebrand, 2005). These gaps exist due to the fact that it can be difficult to track the fleeting behavioural responses of marine mammals to disturbance events under water. Instead reliance is sometimes placed on the demographic effects of disturbances on the populations being studied are identified in addition to or instead of collecting behavioural data (Bowles, 1995; Richardson *et al.,* 1995; National Research Council, 2003; Foote *et al.,* 2004).The responses of marine mammals to sound depend on a range of factors including: the sound pressure level, the frequency, duration, and novelty of the noise source (Antarctic Treaty consultative meeting, 2008). Secondly the physical and behavioural state of the animals may impact how individuals will respond to an auditory stimulus. Finally the ambient acoustic and biotic features of the environment itself may affect how populations/individuals will respond to a stimulus (Hildebrand, 2005). The characteristic of the noise, in particular whether it is continuous or transient, and whether it is constant or changing is an important factor influencing the effect of anthropogenic noise on wildlife. For example, in rodents, exposure to a continuous, intensive sound can result in health effects, while intermittent noises do not (Borg, 1981). This is possibly as a result of the fact that the animals have time to recover between successive exposures to the sound. Humans too have been found to be more sensitive when exposed to a continuous noise than an intermittent pulsed noise (at equivalent peak levels) (Bowles, 1995; Fidell *et al*., 1970). The reasons for this heightened response to continuous noises in mammals, particularly those in an aquatic environment, may be due to the fact that continuous noises have the ability to mask vocalisations for long periods; with an associated drop in the effective range of communication (Bowles, 1995).

It is not only whether a sound is continuous or intermittent that is important. In addition to this, whether a sound is constant or changing is also an important aspect of sound which may influence the behavioural response of an individual. For instance rapid

movements of vessels, with sudden changes in speed or direction, are especially disturbing to marine mammals (Richardson and Würsig, 1997). For example, Californian sea lions (*Zalophus californianus*) are more likely to respond to nearby boats when motor noise levels vary (Richardson *et al*., 1995). In addition to this, hauled-out sea lions are found to show the greatest response to those sea vehicles which make abrupt changes, as these vehicles produce the greatest change in noises (Richardson *et al*., 1995). Myrberg, (1990) supports this argument concluding that a sudden change in amplitude is often considered as a prime stimulus to initiate behavioural responses indicative of disturbance in a species.

With respect to auditory disturbances there are some physiological constraints of animals which affect their ability to respond to disturbances. In pinnipeds, despite similarities in the underwater hearing capabilities of phocids and otariids; there are some remarkable dissimilarities between the two groups in terms of their hearing ranges. Phocids have lower thresholds of hearing at both low (<4 kHz) and high (>20 kHz) frequencies (Kastak and Schusterman, 1998). Additionally, in most phocids, underwater pressure thresholds are comparable to their in-air thresholds, while in otariids, underwater thresholds are higher than their in-air thresholds, which suggest that phocids are amphibiously adapted while otariids have remained essentially air-adapted (Kastak and Schusterman, 1998).

Studies assessing the impacts of noise on animals usually use behavioural avoidance responses as a measure of evasiveness or severity of disturbance (Nowacek *et al*., 2007). This is problematic because motivation and learning can minimise such responses while detrimental effects remain unchanged (Gotz and Janik, 2010)**.** For example, while seals in British Columbia showed a lack of aversive responses to acoustic predator deterrent devices used to protect fish farms (Jacobs and Terhune, 2002) cetaceans were deterred by these devices for several consecutive years (Morton and Symonds, 2002). As the cetaceans did not feed on fish in farms, their motivation to stay in the area may have been lower than that of the seals. This being said, the signals could still have had an effect on the hearing abilities of the seals (Gotz and Janik, 2010); thus, it is important to elucidate the role of motivation and learning in the control of avoidance responses to possible disturbance sources (Gotz and Janik, 2010)**.**

Two decades have passed since the National Research Council (1994) put forward a set of research priorities for understanding the effects of noise on marine mammals. In

most of the areas defined by the priorities a basic understanding is still lacking. The behavioural responses of mammals to noise are complex, and still poorly understood (Richardson *et al*., 1995; Hildebrand, 2005). Responses in marine mammals are thought to depend upon hearing sensitivity, behavioural state, habituation or desensitization, life history traits, presence of offspring, and the type and location of the auditory stimulus. In mammals such as seals, responses have been shown to range from subtle changes in surfacing and breathing patterns, to cessation of vocalization, and avoidance of areas subjected to the highest levels of auditory disturbance (Cassini *et al*., 2004; Hildebrand, 2005). Age and sex are important factors in noise sensitivity. For instance, juvenile and pregnant Steller sea lions (*Eumetopias jubatus*) were more likely to leave a haul-out site in response to overhead aircraft than males and females with young (Hildebrand, 2005).

The heightened level of tourism experienced at these locations can negatively affect marine mammals resting and breeding behaviour if proper management is not in place (Cassini *et al*., 2004). Fences in coastal habitats are expected to dramatically reduce the frequency of human–wildlife encounters by spatially limiting the possibility of human access, provided tourists respect the management schemes. However, because fences function only as physical barriers and do not provide protection against visual/auditory stimuli, to demonstrate their effect fully, it is vital to show not only that human–wildlife interactions are reduced due to a spatial displacement of human disturbance, but also that the behavioural responses of animals change (Cassini *et al*., 2004). Previous studies on pinnipeds underscore the fact that there appears to be a threshold distance (about 10 m between tourists and animals) that triggers negative behavioural responses (Kovacs and Innes, 1990; Cassini, 2001). Cassini *et al*., (2004) study on fur seals found that although human disturbance levels were similar between the years in which the study was conducted, the erection of a fence not only reduced the overall responses to tourists by 60%, but also reduced the behavioural responses of individuals to tourist groups of more than two people, and diminished the responses of fur seals to those tourists which approached at a reduced distance (less than 10 m) (Cassini *et al*., 2004). Although the effects of small and large visitor groups on some species' behaviour have been shown to decrease after the erection of a fence line, the effect that visitor group size has on wildlife has important implications. Studies in this area indicate that visitors should be advised to approach animals calmly, and that larger visitor groups should be split and separated temporally when approaching individuals (Engelhard *et al*., 2001; Ikuta and Blumstein, 2003; Cassini *et al*., 2004).

1.7 Why choose pinnipeds to study disturbance?

Pinnipeds provide an excellent opportunity for researching the impacts of disturbance on aquatic wildlife, as although they spend most of the year at sea, most give birth and wean their pups whilst on land. Pinnipeds have a high predictability in breeding, both spatial and temporally during the breeding season (i.e. they give birth at the same time and place each year). Due to this spatial and temporal predictability it often means that pinniped colonies on land are subjected to high levels of tourism (Pavez *et al*., 2011). The high level of tourism experienced at many breeding colonies has the potential to affect the breeding behaviour of females on the colony which could affect offspring survival. Frequent disturbances of pinniped colonies during the breeding season can alter nursing patterns (Suryan and Harvey, 1999), increase female vigilance (Engelhard *et al*., 2002; Pavez *et al*., 2011), increase aggressive behaviours (Barton *et al*., 1998, Cassini *et al*., 2004) and even cause the abandonment of offspring (Born *et al*., 1999, Cassini *et al.,* 2004). As the survival chance of pups depends largely on feeding in undisturbed nursing bouts throughout the weaning period any disturbance events which reduce the time in which mothers spend nursing their young has the potential to impact on the survival rate of pups at that colony (Drescher, 1979).

1.8 Why choose grey seals to study disturbance?

The grey seal is the largest carnivore native to the UK. The distribution of the grey seal is restricted to three distinct aggregations in the Northern Hemisphere; populations inhabiting the eastern and western Atlantic coastlines, and a separate third isolated aggregation in the Baltic Sea (Harding *et al.,* 2007). Worldwide there are currently thought to be about 380,000 grey seals. On the International Union for Conservation of Nature (IUCN) red list of threatened species, grey seals are listed as being of least concern (IUCN, 2010). This is no doubt in part due to the many laws which have been enforced in order to protect the grey seal populations around the world since the early 1900s. In the UK, under the Conservation of Seals Act 1970, the Natural Environment Research Council is under obligation to the UK government to provide advice on methods to sustain a viable population of grey seals in and around the British Isles (Special Committee on Seals, 2009). Almost 40% of the worldwide grey seal population can be found in the eastern Atlantic subpopulations which inhabit the UK (SCOS, 2011). Whilst around 90% of the UK grey seal populations, located around the Scottish coastline and its surrounding islands and so are isolated from human disturbance, some colonies can be found dotted around the English coastline,

such as the one in Donna Nook, Lincolnshire. These colonies are often exposed to various levels of anthropogenic disturbances. It is important to understand the effect that anthropogenic disturbances have on these populations in order to ensure that viable populations of grey seals are maintained in the future.

Based on prior studies of grey seal behaviour and the responses of other pinniped colonies to sources of anthropogenic disturbance, this thesis will examine the impacts that visual and auditory disturbance events have on the breeding behaviour of the grey seals at a major mainland UK colony subject to high levels of ecotourism. Although much of thesis will analyse the types of behavioural responses derived from anthropogenic disturbance events, the impacts of natural disturbances upon female breeding behaviour will also be examined in this study. This is because it is imperative to decipher whether the range of responses generated by anthropogenic disturbance are both qualitatively and quantitatively different from responses to natural stressors, such as conspecific interactions. The study will not only look closely at any alterations in the reactions to disturbance events as the breeding season progresses, but will more importantly focus on any alterations in biologically significant behaviours such as vigilance and mother-pup behaviours, which have been shown in prior studies to affect the survivorship rates of pinniped pups at breeding colonies (Drescher, 1979; Suryan and Harvey, 1999). The number of individual and environmental covariates may correlate with a females predisposition to respond to a disturbance event, for example, the type and regularity of the disturbance source, the females prior experience of the disturbance stimuli, the abiotic and biotic components of the location within the colony where the female is nursing, the physical and social characteristics of the female and the age, sex and condition of the pup. In order to determine if individual responses to disturbance events are likely to be an indirect result of any of these covariates I tested for an association between the behavioural responses of individuals and the above-mentioned covariates.

1.9 Maternal behaviour and the factors which affect it

After a female grey seal has given birth to a single pup she will quickly establishes a bond with it by learning its unique scent (Redman *et al.,* 2001). This allows identification of the pup within the colony throughout the weaning period (Fogden, 1971; Insley *et al.,* 2003). Although allo-sucking and fostering does occur within the species, females primarily only raise their own single offspring to weaning. Due to the fact that pups face many dangers during the weaning period, females typically stay within a close proximity to their pup to prevent injury (Redman *et al.,* 2001). The

energetic investment into each individual offspring in terms of both pregnancy and rearing are quite high. The reason why grey seals in particular were chosen to be examined in this study was due to the fact that they are capital breeders with a short period of investment (3 weeks) which is very different to most other mammals. The short rearing period to independence of grey seals makes them an ideal study subject as it is possible to follow the full rearing period of a pup in a short amount of time, meaning that during a single breeding season a large sample of females can be followed from birth to weaning. In addition to this, grey seals typically give birth to a single pup meaning there are no litter complications and there is no paternal care within the species. Furthermore as the gestation period for the grey seal is a year, pupping occurs at the same time each year.

Previous studies indicate that the leading causes of pup mortality, excluding still births is from starvation; injuries sustained from conspecifics (Anderson *et al.,* 1979; Baker and Baker, 1988; Redman *et al.,* 2001); being attacked by other species such as gulls, or being crushed or injured during a disturbance event which could lead to the mass movement of individuals (Coulson and Hickling, 1964). In other instances where the pup is injured but does not die, the pup may die at a later date as a result of any infection which may develop in the wounds which the pup sustained from any altercations. Instances where the pup may starve to death include cases where the mother may permanently abandon her pup which may arise due to some form of disturbance event, or by the female purposefully returning to the sea before the pup reaches independence (Coulson and Hickling, 1964; Redman *et al*., 2001). In addition to this, if the mother fails to provide a rich enough milk, in sufficient quantity, the pup may not gain enough mass before reaching independence and will starve shortly after weaning (Coulson and Hickling, 1964, Stevens and Boness, 2006).

The average proximity between the mother and pup varies between sites and pup age and individuals, with females tending to remain closer to younger pups (Boness *et al.,* 1982; Redman *et al.,* 2001). Where topography means it is energetically expensive to reach the sea, or where there is a high likelihood of harm coming to the pup if it is left, then the mother will also remain closer to her pup. On one Scottish island colony, median daily movements to nearby pools of water typically occurred within 10m of the pup (Redman *et al.,* 2001). In short, as the mother is the sole source of nourishment and social interaction that the pup receives before it is weaned; variations in the level of maternal investment are likely to affect the survivorship potential of a pup (Pomeroy *et al.,* 1999). The level of input into these interactions by a female seems to fluctuate

between individuals; with some females showing more attentive behaviours than others (Twiss *et al.,* 2012). A number of factors have been linked to the expected level of maternal input shown by an individual female and these are discussed in detail in the subsections below. In terms of pup survival, any stimuli which have the potential to affect the interactions and/ or bond between a mother and her pup could be detrimental to the offspring's survival.

1.9.1 Maternal characteristics and state dependent factors

Pomeroy *et al.,* (1999) found that a mother's energetic expenditure on her pup in any given year impacts upon her fitness in the subsequent breeding season and excessive expenditure has a cost to the female (Pomeroy *et al.,* 1999). A study by McDonald *et al.,* (2012) noted that life-history theory predicts that selection will favour optimal levels of parental effort that balance benefits of current reproduction with costs to survival and future reproduction; this especially being true of long lived species, such as seals. The optimal level of current reproductive effort will depend on: maternal physiological and behavioural traits; offspring traits; and environmental factors which may affect provisioning. Additionally, how these factors influence effort may differ depending on the stage of reproduction, and any sources of disturbance which may provoke a behavioural or physiological response in the mother, pup, or surrounding members of the colony (McDonald *et al*., 2012). However not only is the correct partitioning of resources important for the female, additionally, the weaning mass of the pups is related to future survival and reproductive success, suggesting that increased maternal energy investment will increase the pups' fitness and chance of survival (Boltnev *et al*., 1998; Hall *et al.,* 2001; McDonald *et al*., 2012). Therefore it is essential that a female partitions her resources between herself and her pup in a way which maximises the chance of her current pup's survival while minimising the negative effects on her potential future reproductive success (Pomeroy *et al.,* 1999; McCulloch and Boness, 2006). Sources of disturbance have the capacity to impact this delicate partitioning of resources.

Bowen *et al.,* (1993) suggested that a mother's prior experience will affect her level of maternal investment; with more experienced mothers investing more into the pre-natal period, and giving birth to larger pups which have higher rates of survivorship (Bowen *et al.,* 1993). Bowen *et al*., (1993) put forward that the reasoning behind this was that older, more experienced mothers should invest more in current reproductive efforts as they may not have many breeding seasons left, whereas younger, less experienced

mothers should invest more in future reproductive efforts rather than investing heavily in the current pup to maximise her lifetime reproductive success (Bowen *et al.,* 1993). A further study by Bowen and Harrison, (1994) reported that the birth mass of the harbour seals on Sable Island, Canada, increased significantly with maternal age, even after the effects of maternal mass and pup sex were statistically removed. Following on from this, it might be suggested that younger females respond more intensely to disturbance events as a result of the fact that they are less invested in their current reproductive efforts.

1.9.2 Pup gender

The debate surrounding whether pup gender impacts on the quality or quantity of care given to a pup by its mother shows no signs of abating; and is not only a is a hotly debated subject in grey seals, but throughout the whole animal kingdom (Anderson and Fedak, 1987; Kovacs, 1987). Within pinniped research there has been some tentative evidence to suggest that females may indeed invest more into male pups than their female counterparts (Anderson and Fedak, 1987; Kovacs, 1987). Most pinnipeds, including grey seals show sexual dimorphism. The grey seal shows the second highest sexual dimorphism of any of the phocid family, behind only that of the genus *Mirounga*; as such adult male grey seals are much larger and heavier than their female counterparts. It can be debated that mothers of male pups may be expected to invest more heavily into rearing a male pup than a female pup; as females tend to have a more or less equal reproductive success regardless of any size differences. This is due to the fact that during any one breeding season most females of breeding age are mated by a male (Amos *et al.,* 1993). On the other hand males have to compete for mating opportunities (Twiss *et al.,* 1998). Typically there is greater variation in males at maturity than females, in terms of size and mating success, and correspondingly, there is a greater variation in the reproductive success rate of males at maturity. Often more dominant males have greater reproductive success than their smaller equivalents; as larger males are often more successful during aggressive bouts with other males when competing with each other to maintain mating access to females on breeding colonies (Twiss *et al*., 1998; Lidgard *et al.,* 2005; Twiss and Franklin, 2010).

Some field studies have supported the theoretical notion presented by Trivers and Willard, (1973) of a differential investment in offspring by females dependent on the pup's gender. For example, on one UK colony, it was observed that mothers of male pups spent more time with their pup, and more time engaged in nursing and defensive

behaviours than mothers of female's pups on the same island (Kovacs, 1987). A further study conducted by Anderson and Fedak, (1987), showed that mothers of male pups had a greater rate of energy transfer to their pups than mothers of female pups; with male pup growth rates 0.36kg^{-1} higher than that of female pups (Anderson and Fedak, 1987). A study by Hall *et al*., (2001) on grey seal pups found that male pups were found to be significantly heavier at weaning, and in better condition than female pups (Hall *et al*., 2001). Hall *et al*., (2001) suggested that high quality females should invest more in male pups because the marginal return, in terms of increased reproductive value, from any additional expenditure was twice that for females (Hall *et al*., 2001).

This being said, there are some studies such as those by Coulson and Hickling, (1964) and by Pomeroy *et al., (*1999), which refute those studies which suggest that pup sex is a determining factor, and argue that pup sex does not influence maternal investment. Coulson and Hickling's, (1964) research indicated that there was an equally important positive correlation between survival to independence and growth rate in both sexes. This study did show that pups had differential growth rates but the study could not attribute these variations to pup gender (Coulson and Hickling, 1964). In addition Smiseth and Lorentsen, (1995a), found that male grey seal pups were born heavier than female pups, but that the growth rates and suckling behaviour were similar for the two sexes.

1.9.3 Environmental Surroundings

The colony and its environmental conditions in a particular year greatly affect the level of investment a mother puts into rearing her pup. Female dispersion patterns on seal colonies are often determined by their pupping site preferences for fine-scale habitat features; primarily access to small pools of water (Redman *et al.,* 2001; Twiss *et al*., 2012, Stewart *et al.,* 2014). As this is the case, pools of water are often a limiting resource on grey seal breeding colonies. From prior studies it is known that females tend to aggregate around pools both for thermoregulatory use and use as a drinking source (Twiss *et al.,* 1994; Redman *et al.,* 2001; Stewart *et al.,* 2014). However a female may choose to pup away from water pools if the pools are already densely populated by other females to save avoidable injury to her pup (Redman *et al.,* 2001). Those individuals who choose to pup further from water sources may remain inactive for longer periods of time to conserve energy and prevent water loss (Harwood, 1976). Redman *et al.,* (2001) suggested that the availability of pools not only impacts on

female metabolic components but an also affects maternal attendance patterns and may have implications for breeding success (Redman *et al.,* 2001).

1.9.4 Evidence for behavioural consistency amongst grey seal individuals

Over recent years the number of papers exploring the notion that personality and consistent individual differences (CID's) in the behaviour of individuals are present across the animal kingdom has exploded (e.g. Smith and Blumstein, 2008; Twiss *et al.,* 2012). Some maternal behavioural parameters have been found to be repeatable in a number of species (Albers *et al.,* 1999; Albers *et al.,* 2000; Weaver *and* de Waal, 2002). Most of this research has focussed on land mammals, birds and fish, with little research until recently being present on marine mammals; however some evidence for the presence of CIDs in both male and female grey seals at breeding colonies in the UK has been unearthed in the past five years. Twiss *et al*., (2011), studied the behaviours of a group of known individuals (both males and female), on the isle of North Rona. In the study, the seals behavioural responses were recorded in response to the release of a novel natural stimulus (wolf sound) at two points during lactation; once at the start of lactation and a second ten to fourteen days later (Twiss and Franklin, 2010; Twiss *et al.,* 2011; Twiss *et al.,* 2012). In grey seals there is some evidence of CIDs, in vigilance type behaviours (Twiss and Franklin, 2010; Twiss *et al.,* 2012). These vigilance behaviours are also key indicators of a response to disturbance events. As this is the case, it is important to check for patterns of individual variation while examining the impacts of anthropogenic disturbance.

A previous study at Donna Nook conducted by James, (2013), indicated the presence of CIDs in some maternal behaviours, within the colony, during the breeding season. However this study did not look at the consistency of behaviours across disturbance contexts. A study by Twiss *et al.,* (2012) at a different colony in the UK reported that whilst CIDs were maintained within a situation, they fell apart when compared over different disturbance situations, indicating that CIDs may be situation specific in grey seals (Twiss *et al.,* 2012).The colony investigated by Twiss *et al.,* (2012) was an isolated island colony which is subjected to very limited anthropogenic disturbance; it would therefore be interesting to see if the seals at the Donna Nook site; which are exposed to higher levels of anthropogenic disturbance also show situation specific CIDs, or vary by showing cross situational CIDs in some behavioural parameters, possibly as a result of habituation to the higher levels of anthropogenic disturbances found at this site.

1.9.5 Behavioural ecology of the grey seal on breeding colonies.

The grey seal is a colonially breeding species. For ten to eleven months of the year grey seals spend their time out at sea or resting at haul out sites; however for eight to ten weeks in the autumn and winter months, grey seals form breeding colonies at predictable locations in the UK (Anderson *et al.,* 1975). In the UK, these breeding colonies usually form on uninhabited beaches, or remote islands which are largely undisturbed, and/ or inaccessible by the general public. The specific timing of the breeding season varies between locations, but is fairly predictable year on year for any given site. Typically each female gives birth to a single pup each year. During the breeding season there is a large turnover of females as individual females will only stay ashore for 18-20 days of the eight week breeding season. Pupping Females and males of the species show high levels of natal breeding site fidelity, and females will often return to the same breeding colony within a few days of previous pupping years (Allen *et al.,* 1995; Pomeroy *et al.,* 1999; Twiss *et al.,* 1994). On a finer scale, females have been shown to have fidelity not only to the breeding colony but to their previous pupping site location within the colony, with females on the Scottish island of North Rona returning to sites within a median distance of 55m from the previous year's pupping site (Pomeroy *et al.,* 1994).

Female grey seals become sexually mature at around three to five years old and breed until they are around 42 years of age (Bowen *et al.,* 2006). Males have a much shorter reproductive lifespan than females; becoming sexually mature at around eight years old and maintaining reproductive activity for around fifteen years (Hewer, 1960; Pomeroy *et al.,* 1999; Twiss *et al.,* 2001). At any one breeding season females will tend to arrive on the breeding colony approximately four days before parturition (Pomeroy *et al.,* 1999). Within this four day window a female will locate and settle into her breeding location within the colony. Quite often females will preferentially give birth in certain locations within a colony; these include areas within the colony which lie closer to pools of water, and areas where there is easy access to the sea (Pomeroy *et al.,* 1999; Twiss *et al.,* 2000a). At the same time, females will often actively avoid areas which are already densely populated by other breeding females (Anderson *et al.,* 1975; Twiss *et al.,* 2000a). It may be possible that intensity and location of disturbance stimuli may impact upon where females will choose to pup on the colony. After delivery of the pup, the females located on colonies which are situated away from the tide line; such as on North Rona and at Donna Nook, will not return to the sea until the pup is weaned; surviving solely on the stored fat reserves present in the thick layer of blubber which

was built up in the months during pregnancy in preparation for this time ashore (Pomeroy *et al.,* 1999). These fat reserves provide the lipid rich food resource that the pup survives on during the weaning period (Pomeroy *et al.,* 1999; Debier *et al.,* 2003). As a result of this highly lipid rich milk provided for by the mother, pups increase weight very rapidly, gaining on average 1.7kg day^{-1} over 18 days; taking their weight from a birthing mass of around 16.5kg to an average weight at weaning of over 40kg (Pomeroy *et al.,* 1999). At the same time as pups increase their mass, the females will lose it. On average females have been shown to lose just under half of their body weight (82kg) during one breeding season (Pomeroy *et al.,* 1999). On North Rona, the efficiency of the weight transferred from the mother to the pup was calculated to be around 45% (Pomeroy *et al.,* 1999); this is a similar transfer efficiency to those noted at other locations, and for other pinniped species (Iverson *et al.,* 1993; Fedak *et al.,* 1996). The high energetic investment involved in rearing a pup to independence, means the rearing period is a critical period for female grey seals in terms of ensuring the pups survival and increasing her own reproductive success. Any occurrences of disturbance within this period that provokes either a behavioural or physiological response from the female or the pup therefore has the potential to be detrimental in terms of reducing both the chances of the pup's survival and the female's reproductive success.

1.10 Study aims

Marine mammals that live near shorelines, such as pinnipeds are potentially at risk from a diversity of anthropogenic processes; and threats to marine mammal populations are expected to increase in severity over the next century (Harwood 2001; Grigg *et al.,* 2012). A key priority outlined by the National Research council report was to define the key behavioural responses of marine mammals to auditory disturbances (National Research Council, 2003). The report also emphasises the need to collect behavioural data in the species' natural environment in order to provide a more profound basis for understanding the potential effects of auditory stimuli on pinniped breeding behaviour (Hildebrand, 2005). Behavioural studies examining the response of breeding seals to human activity, particularly in the context of wildlife tourism, have shown that human activity can result in significant changes in seal behaviour (Cassini, 2001). Assessing the relative importance of environmental and anthropogenic influences on the distribution and behaviour of wild pinniped populations is an important step in designing spatially explicit plans for their management and protection

and it is something that this study hopes to achieve for the Donna Nook site (Grigg *et al.,* 2012)**.**

This projects specific aims were to identify whether various forms of anthropogenic disturbances affected the breeding behaviour of a mainland colony of grey seals on the east coast of England using non-intrusive observational techniques. Whilst behavioural effects from disturbance events have been catalogued for a range of pinniped species, as of yet no studies have identified the effects of human based disturbance events on the breeding behaviour of a mainland colony of grey seals exposed to relatively high levels of ecotourism. This study aims to take a behavioural approach into the effects of disturbance (whilst controlling for other determinants of maternal behaviour, including pup sex, stage of lactation, stage of breeding season etc.), looking at the effects on a wide range of behaviours, not just those associated with mother-pup interactions.

1.10.1 Specific research questions

1. Does the duration that females spend in certain behaviours change between early lactation and late lactation irrespective of disturbance events?
2. a. Do anthropogenic disturbance events impact upon female grey seal breeding behaviour?
   b. What types of aerial anthropogenic disturbances have the greatest impact on female behaviour?
3. Do female grey seals exhibit similar behavioural responses to natural or anthropogenic disturbance?
4. Is there an observable difference in the behaviour of those females which give birth in different locations in the colony? And does the choice of birthing site effect a female's response to a disturbance?
5. Does the pup sex affect female breeding behaviour after a disturbance event?
6. Are there consistent individual differences (CIDs) in behaviour between mothers, in disturbed and non-disturbed conditions?
7. a. How does the quality of mother-pup interactions (MORQ) vary between lactation stages?
   b. Does the mother-offspring relationship quality affect how females respond to disturbance events?
8. What are the immediate behavioural effects of disturbance on grey seals?

2. METHODOLOGY

2.1 INTRODUCTION

This chapter will provide a detailed description of the methodological approaches adopted during the field study. The chapter will begin with an overview of the study site and observation points. The methodology will then move onto to outline the reasons for choosing the Donna Nook site for this particular research project. This will lead onto a precise description of the infield data collection protocols adopted during the field study in order to address the aims outlined in section 1.10.1. Following this a discussion of the data extraction tools and statistical tests adopted to analyse the data gathered in field.

2.2 THE STUDY SITE

2.2.1 Donna Nook, Lincolnshire

The Donna Nook seal colony is one of the few mainland grey seal breeding colonies located in the UK. The site is a 10km stretch of the Lincolnshire coastline on the east coast of the UK, between Grainthorpe Haven in the north, and Saltfleet in the south (53° 28'N, 00° 09' E). Topography of the site is mostly flat with some grass banks and gullies located close to the fence line. After major rain events some silty wallows are present within the breeding colony. When rain levels are high enough, the gullies around the site fill with water, providing areas for seals to drink and swim. This often leads to aggregations of females forming around these areas.

The Donna Nook colony was established in the early 1970s on the Lincolnshire coast and since rapidly expanded from year to year to a peak of 1600 pups being born in the current 2013 season (Abt and Engler, 2009, Lincolnshire Wildlife Trust, 2014). The pup production level at Donna Nook has been increasing by around 15% per year (Thompson and Duck, 2010). Identification of individuals at Donna Nook with flipper tags originating from other breeding colonies, suggests that the growth of the Donna Nook colony may be in part linked to recruitment of individuals from other Eastern Atlantic colonies such as those located in the Farne Islands and on the Isle of May (Pomeroy *et al.,* 2010; Thompson and Duck, 2010). The section of the breeding colony which is open to members of the public is frequented by an estimated 70,000 visitors per breeding season (Thompson and Duck, 2010). Mother- pup pairs located along this public stretch of the beach are exposed to both visual and auditory stimuli from visitors. Disturbances by visitors fluctuate in intensity but are a constant occurrence during daylight hours. The high visitation level experienced at Donna Nook, along with

the occurrence of military exercises by the RAF during the breeding season, provides an ideal opportunity for the assessment of the impacts of human disturbance on maternal behaviours at the site throughout the lactation period. Public access to the colony is restricted to a designated footpath with a countryside type fence erected in 2007 to prevent direct seal-human contact. These fences are patrolled by volunteer wardens from the Lincolnshire Wildlife Trust. The Volunteers at the site ensure visitors do not get too close to the seals; and also provide a means of education the general public on the grey seal breeding cycle, and the effects that humans can have on wildlife when we invade their natural habitats.

The site itself has been managed by the Lincolnshire Wildlife Trust for a number of years. The land is owned by the Ministry of Defence (MOD) and part of the site is still used for target and bombing practise. Only a small section of the site, located close to the town of North Somercotes was used to collect the data for the study. The stretch where data collection took place was an access path open to the public which had been fenced in 2007 in order to reduce the level of interactions between the seals and the general public. Access to the public site was obtained in two areas: the public access entrance from the Stonebridge car park and a public access point through a farmer's field from behind the site (Figure 2.1).

2.2.2 The site and research into anthropogenic disturbance

The main aim of this thesis is to discern whether anthropogenic disturbances effect grey seal breeding behaviour. The Donna Nook site provides an ideal location in order to observe the possible impacts that anthropogenic disturbance events may have on female grey seal breeding behaviour. The site is widely regarded as the location to which members of the general public can get the closest encounters with seals in their natural environment in the UK; with people being able to get as close as 1-2m to the seals. The site attracts a large number of people from a broad range of socio-economic backgrounds; from school groups, to wildlife photographers, to family outings; all of which will have different expectations and knowledge about the animal which they are viewing. As such the site is an ideal choice on which to carry out research into the effects that these close encounters with the general public have on the breeding behaviour of the seals. Management schemes set up by the Lincolnshire Wildlife Trust have prevented direct interactions between the seals and the public by erecting a fence along the public path in 2007. No path is present on the RAF site, although there is restricted access to this site.

Part of this thesis involves looking into the possibility that aerial disturbances may affect the breeding behaviour of grey seals (refer to aim 2a in section 1.10.1). In addition to the pedestrian effect on the seals, the Donna Nook site provides an ideal place to research the effect that aerial disturbance events have on the breeding colony. From previous studies conducted by Tracey and Flemming, (2007), it has been shown that in some mammal species, aerial disturbances have behavioural effects on the species being studied (Tracey and Fleming, 2007). As of yet no research has been done to see if aerial disturbances have any effect on grey seal populations whilst they are on land breeding. The Donna Nook site is situated on the property of the Ministry of Defence (MOD), with the breeding colony situated on an active RAF base, thus providing the perfect environment in which to test whether aerial disturbances affect grey seal breeding behaviour. The Donna Nook base frequently carries out target and bombing drills using overhead aircraft. Although no bombing drills are permitted to go ahead during the breeding season, target practice over the breeding colony still goes ahead. Due to the variation in the type and durations of flyovers; with both fixed wing and helicopter fly overs occurring during the breeding season, the site provides an ideal location in which to study the effects that differing types of aircraft have on the behavioural responses of seals.

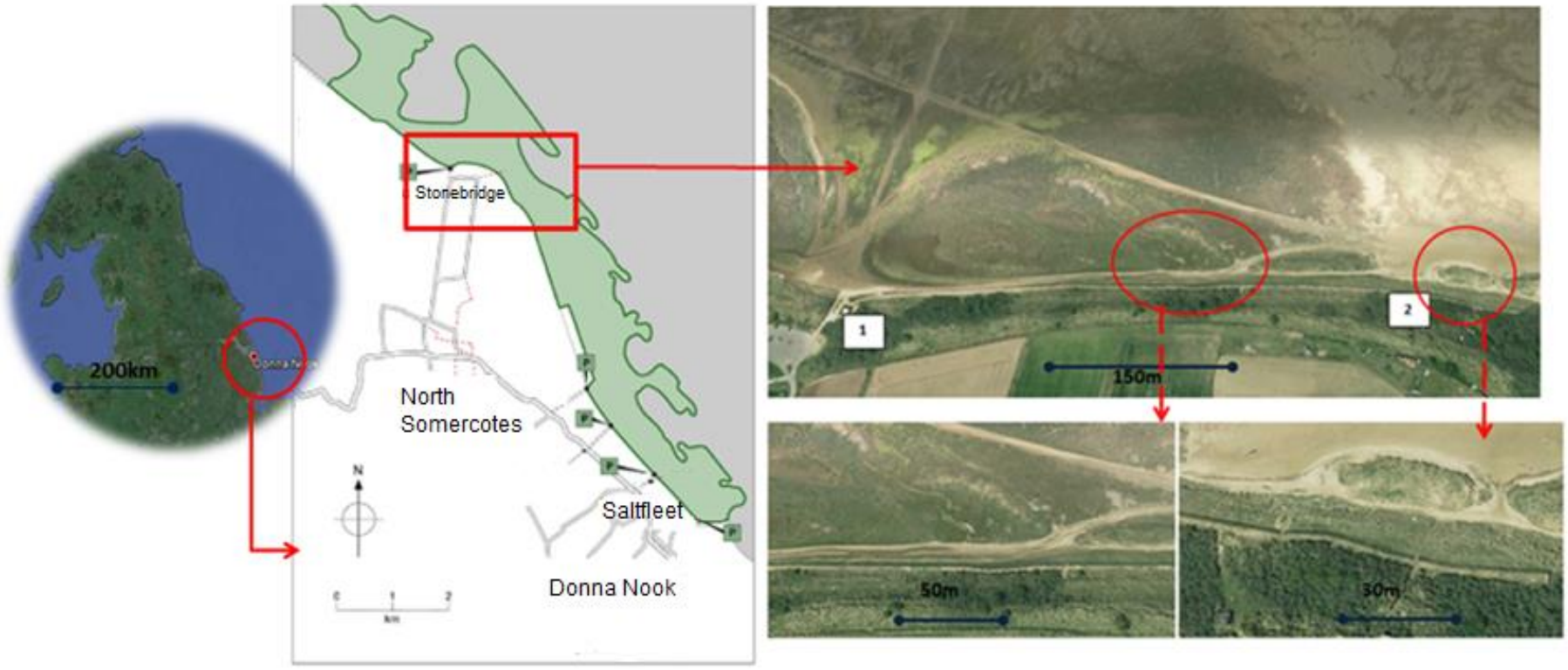

Figure 2.1 Map indicating the location of the Donna Nook Nature Reserve area along the eastern UK coastline. The image is available from http://lincstrust.org.uk/reserves/nr/print-reserve-maps.php?mapref^{m}15. Satellite images taken from Google Earth depict the public site in greater detail, with close up views of the two observation sites where behavioural data was collected. The two numbered areas are the two access points for the general public; '1' being Stonebridge car park and '2' being the access point from an adjoining farmers field.

2.2.3 The observation dates and locations

Observations took place between sunrise and sunset on the 28th of October until the 5th of December 2013. Observations were taken between the hours of 7:00am and 4pm due to the fact that there was an observable difference in visitor attendance patterns between these hours, which allowed behavioural data to be collected both in the presence and absence of visitors to the site. Observations were made from two areas along the public access pathway at the Donna Nook public site. The first of these was close to the Stonebridge car park and the other was close to the Ministry of Defence (MOD) site (**Figure 2.1**.). From here on in, when the study refers to the RAF site, it refers to the location on the public site which borders the MOD property (site 2 on **Figure 2.1**) and when the study refers to the car park site it refers to site 1 on **Figure 2.1** which borders the Stonebridge car park. Observations were made on foot behind the public fence. In order for accurate and representative focals to be taken, correct positioning of the observer was crucial. Where possible, one position was taken for the entire day in order to reduce any disturbance to the animals via movement of the equipment to new locations. In addition to this, the observer where possible, was located downwind of the focal individual as to reduce the impact that novel smells from the observer may have had on the study animals. The fence was never crossed at the public site in order to ensure that the observer did not contribute to the level of disturbance, and to ensure the safety of the observer. Observations took place for the majority of the breeding season in order to assess whether individuals responses to disturbance varied as the season progressed.

2.3 FIELD IDENTIFICATION TECHNIQUES

2.3.1 Female Identification

In order to answer the specific aims outlined in section 1.10.1 it was of paramount importance that individuals in the field could be identified in the field in order for repeat observations throughout lactation to be recorded. Sixty one females were uniquely identified as part of this study. The selection process of choosing individuals composed of two principle considerations. Firstly it was imperative that the female had preferably a stage one pup (see section 2.3.2); although a few early stage two pups were included in the study, particularly towards the end of the breeding season where the number of stage one pups close to the fence line was limited. This selection criterion was vital in ensuring that the mother's change in behaviour throughout lactation could be recorded. The second consideration was the appearance of distinguishable features which would

make correct re-identification of females throughout lactation possible. Distinguishable marks included distinctive pelage patterns on either, or both of the flanks, scars or other injuries (**Figure 2.2).** The females with the most distinguishable marks within the study area were favoured, and all females were selected based upon this criterion.

Figure 2.2: Some examples of distinguishing marks and scars which were used to identify individuals in order to get repeat observations of individuals.

Once a female had been selected as a subject, the individual was given a seal ID in the field. The prefix "P" of the ID related to the fact that the study was conducted at the public site, and the suffix number of the ID corresponded to the order in which the females were first discovered; e.g. P29 was the 29th female recorded in the study which was conducted at the public site.

In the field, photographs of each female were taken using the Canon 40D body and a Canon EF 70-300mm f/4-5.6 IS USM lens; with special attention given to capturing photographs of the two flanks of the individual for identification purposes. Photograph numbers, along with the corresponding seal ID were recorded in a notebook for later referral. Photographs were later collated into a photo-catalogue for each individual. These photographs were stored on an Acer Laptop (Model: Acer Aspire 5741). Another copy of the clearest photographs was stored on an IPhone 4s (Model: A1387; Version: 7.0.4 (11B554a)) for reference purposes in the field.

Using the aforementioned identification techniques, females could be quickly and more importantly correctly re-identified at later points in the lactation period. Noting the location of individuals in the study area also aided with the re-identification of individuals. This is due to the fact that females do not tend to move far after the birth of their pup (Pomeroy *et al.,* 1999; Redman *et al.,* 2001).

The correct re-identification of individuals during the study was imperative in determining whether individuals behave consistently when presented with a particular disturbance event. Repeats observations also allowed the study to answer aim one outlined in section 1.10.1, this being whether the females point in lactation affected how an individual may respond to a disturbance event. Where possible, photographs were taken at every re-sighting event in order to match them to prior photographs and sketches taken of the animal. Photograph matching from different sighting were done by eye and were achieved by matching distinguishable marks or scars on the flanks/ face of the individual. Where photographs could not be taken, the video focal was viewed at a later date and paused when a clear flank shot was achieved. This shot was then compared to prior photographs of the individuals to make a firm confirmation of the ID. The retrieval of behavioural information from video files only took place after confirming all the IDs of individuals from the focal videos after field work collection took place. It must be noted this study made no attempt to assess identification error rate (refer to section 4.4.1c).

2.3.2 Pup Stage Classification.

During the study pups were classified into five discrete developmental stages. These corresponded to the stages outlined by James, (2013); the principal characteristics of which are outlined below:

Stage one (Figure 2.3): Yellow tinge to lanugo; fresh red/ pink umbilicus; ribs and pelvis visible; lacks coordination or much movement. Approximate age is 1-3 days

Stage two (Figure2.3): Lanugo is white; umbilicus is either lost completely or has darkened to a brownish colour; pelvis and ribs are less visible as blubber develops; some co-ordination of movement has developed. Approximate age is 4-8 days.

Stage three (Figure2.3): Possibly some loss of white lanugo coat on the extremities (i.e. flippers and muzzle), however main body will still be full white lanugo; body is barrel shaped with increasing blubber mass; good, sound co-ordination. Approximate age is 9-14 days.

Stage four (Figure 2.3): Moulting of the full body lanugo has begun although not complete; mother will have been/ or just about to be mated. Approximate age is 15-17 days.

Stage five (Figure 2.3): Full moult of lanugo coat to juvenile pelage; typically the pup is weaned. Age of pup is 18+ days old.

Figure 2.3: Different pup stages at the Donna Nook site: A and B are Stage 1 pups (notice the yellowing colour of the Lanugo coat and the loose folds of the skin in B). C and D are Stage 2 pups (note the darkening colour of the umbilicus in stage two pups). Pictures E and F are of stage 3 pups (notice the rotund shape and some loss of the Lanugo coat on the extremities). Image G is a stage 4 pup (some loss of Lanugo on the body) and image H shows a Stage 5 pup. At this stage the pup is likely weaned from their mother

2.3.3 Pup Sex Determination

Pup sex determination was crucial in answering question 5 outlined in section 1.10.1; this being whether pup sex affected female breeding behaviour, both in the presence and absence of disturbance events. Where possible the observer tried to determine the sex of the pup. The sex of a pup can be determined rather simply by distinguishing whether there is presence, or absence of a penile opening. This is identified by a small opening on the ventral surface midway between the navel and the hind flippers (**Figure 2.4**). This opening is more easily identifiable to observers when the pup reaches a stage 3 size, as the pups have gained a substantial amount of weight and appear bloated and barrel like in shape making the penile opening visible. Visibility of the propice is not restricted o stage III or above however at earlier stages, the pup is less mobile and folds of loose skin obstruct the view of this opening making sex determination at this early stage more difficult. As handling of the pups was not permitted at the site, pup sexing were not always possible. This was due to the fact that pups often orientated themselves with the ventral surface on the ground or towards their mother, meaning the view of their ventral surface was obscured from the observers view. In addition to this failure to see the propice is not certain proof that the pup is female. In this respect it is important to keep in mind that remote sex determination is uncertain, however as hands-on techniques were not permitted at the site, remote identification was the only solution.

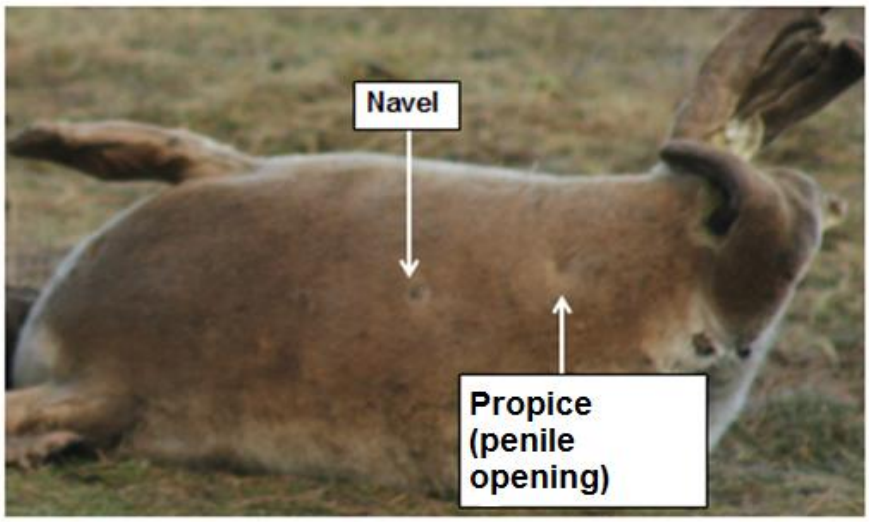

Figure 2.4: Sex determination in pups. A penile opening between the navel and the flippers would indicate that this individual was a male. Sexing is generally easier once the pup has reached a stage three size where the pup appears to be bloated in shape.

2.4 DATA COLLECTION PROTOCOLS

Data Collection took place in the 2013 grey seal breeding season; between sunrise and sunset on the 28th of October until the 5th of December 2013 at the Donna Nook site in Lincolnshire. Data collection protocols consisted of focal videos, proximity mapping, environmental/disturbance measures, behavioural observations, and the measurement of in-field sound levels (amplitude (dB)). This projects aim was to uncover the effects that anthropogenic disturbances have on mothering behaviours across lactation. It was imperative that repeats were collected for each female, preferably in early and late lactation, and at times where there was little to no disturbance and also at times of high disturbance. Early lactation will be defined as mothers with stage one and stage two pups and mothers with stage three and stage four pups are referred to as being in late lactation. These lactation stages are based on the classification of lactation stages described by Iverson *et al.,* (1993).

Comparisons of behaviour from the focal videos chiefly relied upon comparing the percentage of the focal (POF) individuals spent in certain behaviours. The POF that individuals spent in each behaviour was calculated for each focal. Females typically had at least four focals (two in early and two in late lactation) and the POF that each individual spent in each behavioural category were calculated for all focals. POF was used instead of daily activity budgets due to the fact that each focal only lasted for around half an hour and so did not comprise of a significant proportion of the individuals day, making inferences about activity budgets from this period unreliable and most likely, inaccurate. POF was adopted over scan sampling techniques due to the often short lived nature of disturbances and the behavioural responses to disturbances in individuals.

2.4.1. Focal Videos

In order to address the research questions outlined in section 1.10.1., focal videos were used to record the behaviours of chosen individuals at early and late lactation stages. The focal videos were taken using a Panasonic (model: HC-X920) camcorder. Each video focal was approximately 30 minutes long. Thirty minutes was deemed suitable based on several reasoning's, these being: the methodologies of previous studies (e.g. Twiss *et al.,* 2000; Twiss *et al.,* 2012); the results of prior work undertaken at the Donna Nook site (James, 2013); and the results of trial studies undertaken on the 26th and 27th of October 2013 at the Donna Nook site. There are some limitations to

using these short sampling times, and these will be discussed in the general discussion chapter.

Focal videos have been used in a number of behavioural studies from a range of taxa, such as Aves (Nakagawa *et al.,* 2007), to land mammals such as primates (Lee, 1984; Weaver and De Waal, 2002) and even marine mammals including both cetaceans (Hill *et al.,* 2007) and pinnipeds such as the grey seal (Twiss *et al.,* 2011; Twiss *et al.,* 2012). Although scan samples have previously been used in many studies to create behavioural time budgets (Twiss *et al.,* 2000; Twiss and Franklin, 2010; Anderson *et al.,* 2011), due to the random nature of disturbance events scan samples would not be suitable for this study. In addition to this, many important seal breeding behaviours such as pup checks are instantaneous in nature, lasting for only a brief time frame; and so would be missed by scan sampling techniques. Using focal videos to study individuals allows these brief behaviours; which are biologically important, to be incorporated into evaluating the fine scale reactions of behaviour to disturbance events.

Initial behavioural observations were taken from females that were identified as having pups classified in early lactation, preferably stage one pups. If possible, for each female, two observations were carried out at early lactation, one with little/ no disturbance events and one with more frequent disturbances. The same two videos were also collected during late lactation for each female. This was not possible for all focal females in the study; as the nature of many of the disturbance events located at the site meant that they were often unpredictable and unrepeatable. After approximately fifteen days a second cohort of females were selected. These females were chosen, not only to increase the overall sample size of females in the study, but also to gather information on the variation in behavioural responses to disturbances between those mothers which bred towards the start of the breeding season, and those that bred towards the end of the breeding season.

Focal videos were taken between the hours of 7am and 4pm. Individuals that were to be observed that day were first identified. The order in which the focals were taken were based on a prior methodology adopted by Altmann, (1974) in which the first focal was the female which approached her pup first. This methodology ensured randomisation in the sampling method (Altmann, 1974). Using pre-determined behavioural categories in the form of an ethogram (see section 2.5) the focal videos were then analysed at a later date in order to extract the data pertinent to the study.

2.5 EXTRACTION OF BEHAVIOURAL AND DISTURBANCE DATA

2.5.1 Extraction Protocol

Extraction of behavioural data commenced in February 2014; post field season. For extraction of behaviours from the videos to commence, an ethogram of behavioural categories was created. In order to construct this ethogram, a number of published ethograms from previous studies on grey seal and related species were consulted (Wilson, 1974; Kovacs, 1987; Twiss *et al.,* 2011).These ethograms from prior studies were combined with observations that were noted in the field during the field season. A fine scale description of the behavioural categories used in the finalised ethogram can be found in **Table 2.5**

Once the ethogram categories had been finalised, a Visual Basis for Application (VBA) programme was created in Microsoft Excel (Microsoft 2010; **Figure 2.5**). Each behavioural category from the ethogram was given a unique icon, which when selected logged the behaviour and the time of logging of the behaviour on excel. When a subsequent behaviour was then performed by the focal seal, the button for that behaviour was then pressed, providing an end time to the last behaviour and a start time for the next behaviour. Having the start and end time of the behaviours performed by an individual over the video focal allowed the duration that individuals spent in certain behaviours in the focal before and after disturbance events to be calculated. All video focals were processed in this way.

After behavioural data extraction from the videos was complete, for each focal the total duration that the female spent in each of the behavioural categories was calculated. Then from this, the percentage of each focal (POF) a female spent performing each of the behavioural categories was calculated. The POF that the seal spent in each of the defined behaviours was calculated using R programming software (http://www.r-project.org/).

Disturbance event information was used throughout the analysis in correspondence with data collected from the complementary focal videos. A separate VBA programme was constructed in Microsoft Excel 2010 in order to extract disturbance data which may have been missed while collecting the data in the field (**Figure 2.6**). Using a VBA also permitted a more accurate estimation of the duration of each disturbance event. For a description of the disturbance categories used in this VBA please consult **Table 2.6.** In instances where two disturbances occurred at the same time; both of their durations,

amplitude levels, and locations with respect to the focal female were noted in field. While watching the videos, instances where two disturbances occurred simultaneously were rare. Where two disturbances did occur simultaneously, the disturbance which caused the biggest change in amplitude (dB) was recorded.

2.5.2. Behavioural and disturbance categories

2.5.2a Behavioural Categories

In order to answer the questions outlined in section 1.10.1 it was imperative to discern the behaviours that might be key indicators of a response to a disturbance event. The key broad behaviours that made up the behavioural ethogram used in this study were resting, comfort movements, active movement, mother-pup behaviours, threatening behaviours, nursing, aggression, copulation, pup checks and alert behaviours. These broad category behaviours were chosen based on the indications from prior studies that the frequency/ duration of these behaviours have previously been altered in some megafauna species which are subjected to anthropogenic disturbances (Cassini *et al.,* 2004; Hildebrand, 2005; Tracey and Flemming, 2007; Anderson *et al.,* 2011; Jiang *et al*., 2013). In the behavioural ethogram outline in **figure 2.5** it is observable that these broad behavioural categories have been broken down in to more fine scale behaviours. This was done as a result of the completion of prior video analysis from previous breeding seasons at the Donna Nook site which suggested that individuals showed a very minimal response to disturbance events. From this analysis it was hypothesised that there may be still small discernible behavioural responses to disturbance events such as change in the amount of time females spend alert with their head down or alert versus alert with their head up. It was from this hypothesis that the broad behavioural categories were broken down into the more fine scale behaviours outlined in **figure 2.5.** Analysis after behavioural extraction from the ethogram indicated that these fine scale behavioural categories were largely unnecessary and produced no different results than those obtained for the broad scale behaviours. This is why the statistical analysis, almost solely concentrated on analysing the amount of times females spent in the broad behavioural categories.

Vigilance behaviours are often a key behavioural parameter associated with responses to external stimuli. Disturbed animals will often undertake vigilance behaviours to evaluate the potential danger to themselves and possibly their offspring (Dyck and Baydack, 2003; Cassirer *et al*., 1992). In this respect, it is possible that measuring the vigilance responses of individuals in disturbed populations could be a useful way in

which to measure the effects of disturbance events on targeted wildlife populations. In grey seals the two main types of vigilance response are alert and pup checking behaviours. During initial field observations it was noted that females sometimes perform alert behaviours with their head down and other times with their head up and so it was decided to distinguish between these two behaviours in the VBA in order to look for any differences between head-up alert (HU Alert) and head down alert (HD Alert) behaviours during disturbance events. Similarly pup-checking behaviours could be separated into the classic pup check where the female clearly raises her head off the ground and looks at her pup (PC) or those where she keeps her head on the ground but makes direct eye contact with her pup (pup glace: PG). Pup glances could only be noted in focals where the female faced the observer.

Nursing behaviours are often cited as being one of the key behavioural parameters to be affected by disturbance events (Kovacs and Innes, 1990; Phillips and Alldrege, 2000; Anderson *et al.,* 2011). As the survival chance of pups depends largely on feeding in undisturbed nursing bouts throughout the weaning period, any disturbance event which reduces the time in which mothers spend nursing their young has the potential to impact the survival rate of pups at that colony (Drescher, 1979; Kovacs and Innes, 1990); as such it was felt pertinent to include nursing as a behavioural category in this study. Upon watching videos of female grey seals in previous years it was noted than females switched between two types of nursing, nursing whilst resting (R Nurse) and nursing while alert (A Nurse). As these two combinations appeared to be distinct behaviours in their own right it was decided to include the two types of nursing behaviour in the VBA (see **Figure 2.5**). It is important to note here that this study recognises that the behavioural appearances of nursing does not necessarily equate with the amount of milk transferred as the pup will often detach itself from the nipple during ‘nursing’ bouts (Pomeroy *et al*., 1999).

Resting behaviours are important for any species, but for grey seals, resting is particularly important during the breeding season due to the fact that they are capital breeders (Cassini *et al*., 2004). The heightened level of tourism experienced at these locations can negatively affect their resting and breeding behaviour if proper management is not in place (Cassini *et al*., 2004; Osinga *et al*., 2012). Certain behavioural responses, such as a decreased amount of time spent in resting behaviours in response to human disturbances can be used to estimate tolerance, which can then guide management of natural areas at local and regional scales (Gill *and* Sutherland 2000; Fernandez and Telleria*,* 2000).

Further behavioural categories that were explored in this analysis were: movement behaviours, separated into movement to, and away from the pup, and within and beyond 2twobody lengths of the pup (LocA2+, LocA2-, LocT2+, LocT2-); comfort behaviours (separated into comfort movements (CM), drinking and exploration); copulatory behaviours (ATCOP, COP); aggressive behaviours (separated into threat and physical aggression (PhysAgg); and finally behaviours centred around female-pup interactions. These mother-pup interactions were separated into positive mother-pup interactions (Presenting, smell, MP-Int, flipper defence (FP Dfnc)) and negative mother-pup interactions (rejecting pup contact (RPC) (**Figure 2.5, Table 2.5**).

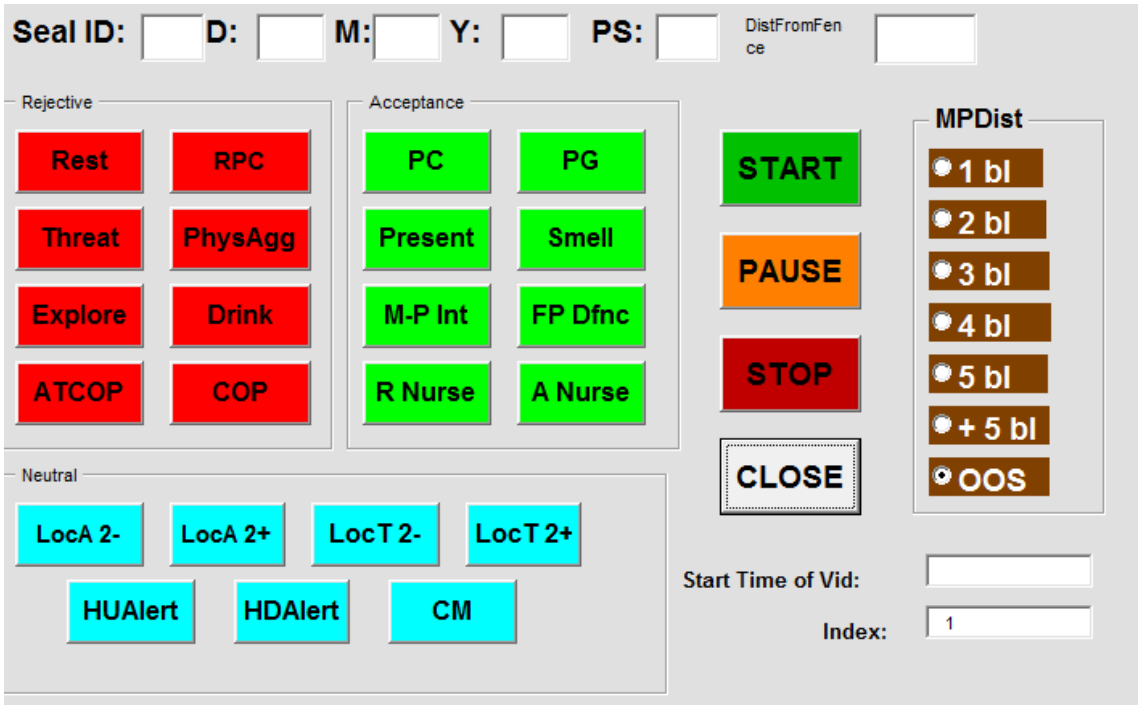

Figure 2.5: Screen shot of the data entry window used in Microsoft Excel. This VBA macro was used to extract behavioural data from the video focals that were taken in the field. See **Table 2.5** for definition of terms.

Table 2.5: Ethogram describing the behavioural categories used in the analysis of the impacts of disturbance on individual behaviour. The column labelled MORQ categorises behaviours according to whether they were included as affiliative behaviours (**A**) or rejection behaviours (**R**) when calculating Mother – offspring relationship quality index (MORQ). Blank means that the behaviour was not included when calculating the MORQ index before and after disturbance events. See section 2.9.1 for a discussion of MORQ and justification of MORQ categories

Behaviour (and brief code)	Description	MORQ (Rejective (R) and Affiliative (A)	Broad Behavioural Category (*more information see 2.9.1)
Rest (Rest)	The female is in a relaxed state with her head on the floor. She is not engaging at all with her pup.	R	REST
Drink (Drink)	The female dips mouth into water and appears to intake water	R	COMFORT
Exploration (EX)	Nosing of the terrain without looking up. No movement by the female was recorded.	R	COMFORT
Reject Pup Contact (RPC)	The pup attempts to make contact with the female. The female responds by retracting or moving her body away from the pup.	R	MPBEH
Threat/ aggression (THREAT)	Any form of aggression that involves non-physical violence directed at other individuals by the focal female. Includes open mouth threats, howling or hissing.	R	THREAT
Physical Aggression (Phys Agg)	This behaviour includes aggression directed by the female at a conspecific. Contact must be made between the two individuals involved. One of the individuals in the physical altercation must be the focal female	R	THREAT
Attempted Copulation (ATCOP)	Unsuccessful copulation. Male must make some attempt to mount female. A male approaches and grips female. He may place his body over hers to begin with. Intromission is not achieved.	R	COPULATION

*Continued Overleaf

Copulation (COP)	Only successful copulation attempts. A successful copulation is when intromission is achieved.	R	COPULATION
Presenting (PR)	The mother lies on either flank to expose her nipples to the pup. Presenting had to involve the pup nosing the nipple. This was to eliminate instances where the female was just resting on her side. Flippering the pup down to the nipples is included in this behaviour	A	MPBEH
Rest Nursing (RN)	The pup makes contact with the nipple and begins suckling. During rest nursing the mother must be lying down with her eyes closed.	A	NURSING
Alert Nursing (AN)	The pup makes contact with the nipple and begins suckling. During alert nursing the mother must be either lying down with her eyes open and actively looking around or with her head raised above the ground	A	NURSING
Pup-glance (PG)	The mother does not lift her head but makes a directional look at her pup	A	PUP CHECK
Pup Check (PC)	The mother lifts her head to make a direct aimed look at the pup	A	PUP CHECK
Smell (SM)	The mother presses her nose against any part of the pups body	A	MPBEH
Mum- pup interaction (M-PINT)	Includes female rolling to touch her pup and interactive play behaviours	A	MPBEH
Flipper Defence (FP Dfnc)	Mother flippers pup to move the pup away from interfering hetero- or con-specifics. This is usually followed by some form of aggressive interaction towards the disturbance source	A	MPBEH
Locomotion away from pup (<2bl) (locA 2-)	The mother either rolls or uses or fore flippers to move away from her pup whilst being 2bl (bl=body length) or less away. The movement must have some		MOVE

*Continued overleaf

	purpose. This can include the onset of aggressive behaviours		
Locomotion away from pup (>2bl) (LocA 2+)	The mother either rolls or uses or fore flippers to move away from her pup whilst being at least 2bl away from her pups location. The movement must have some purpose. This can include the onset of aggressive behaviours		MOVE
Locomotion toward pup (<2bl) (LocT 2-)	The mother rolls or uses her fore flippers to move towards her pup whilst being under 2bl away from her pup. This includes any movement in the onset of aggression		MOVE
Locomotion toward pup (>2bl) (LocT 2+)	The mother rolls or uses her fore flippers to move towards her pup whilst being at least 2bl away from her pup. This includes any movement in the onset of aggression		MOVE
Head Up Alert (HUALERT)	The female lifts her head to look around at her environment. This can be a directional look at a disturbance source or a non-directional scan of her surroundings. This does not include an intentional look at her pup (see Pup Check)		ALERT
Head Down Alert (HDALERT)	The female is in contact with the ground but has her eyes open. This can be a directional look at a disturbance source or a non-directional scan of her surroundings. This does not include an intentional look at her pup (see Pup Check)		ALERT
Comfort Move (CM)	The focal female scratches, shakes or adjusts the position of some part of her body.		COMFORT
Distance	The distance between mother and pup in seal body lengths (1bl≈2m)		

2.5.2b Disturbance Categories

A detailed collection of disturbance data was essential in order to answer questions 2-8 outlined in section 1.10.1. Although this study chiefly considered the effect that anthropogenic disturbance events have on grey seal breeding behaviour; in order to answer question 3 defined in section 1.10.1., regarding whether natural disturbances effect breeding behaviour in a similar way to anthropogenic disturbances it was essential that natural disturbance events during the focals were also recorded. With regard to anthropogenic disturbances, the Donna Nook site is unique in terms of how close visitors can get to breeding seals (often with 1-2m) and also its close proximity to the activity ministry of defence (MOD) site which undertakes fly-overs over the colony throughout the breeding season. At the Donna Nook site there are a number of forms of aerial disturbances that the seals are exposed to from the RAF site including different forms of helicopters and jets. In addition to this, there are also non-military, commercial jet aircraft flyovers over the colony from a nearby airport. The commercial aircraft are smaller in size than the military aircraft, are located at a much higher altitude than military aircraft (approximately 30,000 feet compared to the military aircrafts 10,000 feet) and correspondingly the military aircraft produced much louder sounds than the commercial aircrafts (approximately 100dB compared to around 65-70dB for commercial). As the two sites examined in this study were very close to one another (within 0.5km) the effects of aircraft disturbances were more or less identical. Peak amplitude levels for each type of disturbance were fairly consistent between the two sites, with no significant differences in amplitude observed for any disturbance category. The sound meter used in this study did not record frequency; as a result the frequency of noises could not be measured in this study.

Previous studies have indicated that both pedestrian and vehicular anthropogenic disturbances can elicit behavioural responses from megafauna species, with different species eliciting different responses to similar disturbance triggers (Cassini *et al.,* 2004; Tracey and Flemming, 2007; Antarctic Consultative Meeting, 2008). Although it may be thought that aerial or land vehicles may provoke a greater response than pedestrian approaches, in fact many studies have revealed that pedestrians evoked a greater response by individuals within a population than other anthropogenic stimuli (land/ aerial vehicles) (Stankowich, 2008; Cassini *et al*., 2004). Due to the different behavioural responses elicited not only by different species but by different populations of the same species to similar disturbance events, this study opted to split the four broad behavioural categories of pedestrians, aircraft, aggression and natural

disturbances into more fine scale categories. This was done to establish whether certain types of pedestrian/ aircraft/ aggressive/ natural disturbances evoked a behavioural response. The fine scale categories were established from observing the site for two days before collection protocols commenced. For a detailed look at the fine scale disturbance categories used please refer to **Table 2.6.** As with the behavioural categories, analysis after data collection revealed that the results gathered from the fine scale and broad scale categories were very similar and so it was decided that, apart from the analysis of aircraft type on behaviour, all behavioural analysis in respect to disturbances would use the four broad behavioural categories. These four disturbance categories were: disturbances that originated from pedestrians; disturbances that originated from aircraft; disturbances from conspecifics; and disturbances originating from other natural sources excluding the weather.

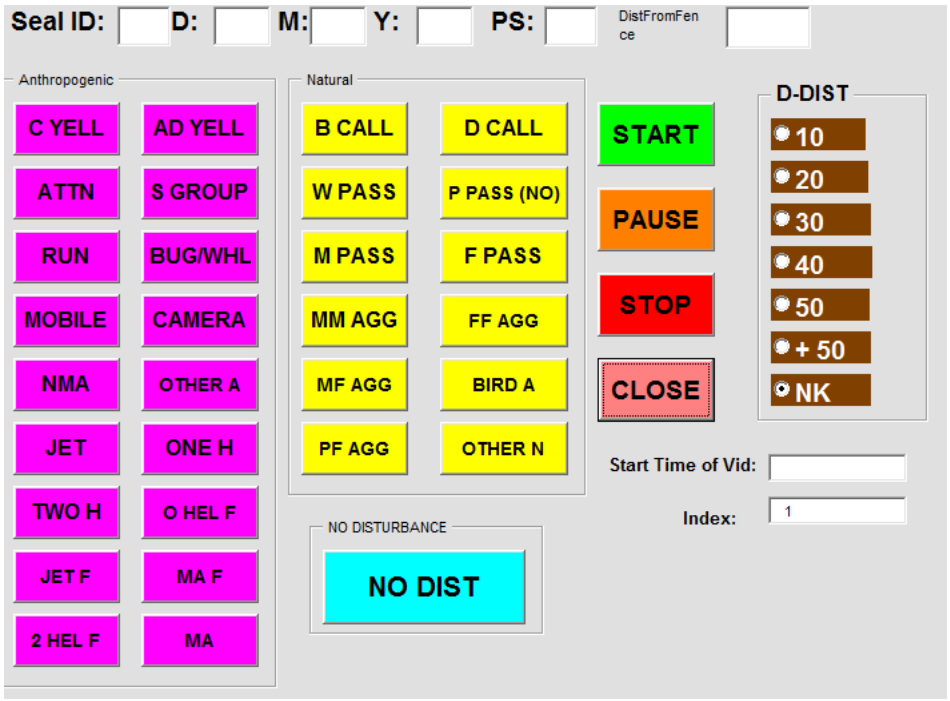

Figure 2.6: Screen shot of the data entry window used in Microsoft Excel. This VBA macro was used to extract additional disturbance data from the video focals that were taken in the field to supplement any notes which were taken in the field. For definitions please see **Table 2.6**

Table 2.6: A categorisation of the disturbance events which were recorded using a VBA macro. A description of each of the disturbances is listed along with its categorisation as either an anthropogenic (A) or natural (N) disturbance and which broader disturbance category they each fell in. Broad Disturbance categories are; pedestrian (P), Aircraft (AIR), aggression (AGG) Natural (N)

Disturbance	Description	Anthropogenic (A) or Natural (N)	Broad Disturbance category
Child Yell (CYELL)	A child screaming either at the seal or at another person. Includes crying	A	P
Adult yell (AD YELL)	An adult screaming at either a seal or another member of the public. Also includes laughing or crying.	A	P
Attention noise (ATTN)	Any noise which is purposefully made by members of the public to try and get the seals attention. It does not have to be the focal seal. Includes whistling, clicking or other behaviours of a similar nature.	A	P
School Group (SGROUP)	Any disturbance caused by the presence of a school group at the study site. This includes any shouting, running, attention grabbing behaviour performed by the group.	A	P
Running (RUN)	Any noise from the video which may indicate a person has run on the gravel across the path	A	P
Buggy/ wheelchair (BUG/WHL)	Noise of any buggy/ wheelchair moving across the gravel along the path. Includes motorised scooters.	A	P
Camera (CAM)	Visual/ auditory disturbance associated with the presence of any camera equipment. Includes any instances in which a flash is noticed	A	P
Mobile (MOBILE)	Any instance where a mobile phone rings at the site	A	P
Other Anthropogenic	Any novel disturbance events which has not been accounted for	A	P

*Continued Overleaf

	in the descriptions above which had an anthropogenic origin.		
Non Military Aircraft (NMA)	The passing of a non-military aircraft flying over the colony.	A	A
Jet (JET)	Any auditory/ visual disturbance associated with a military jet crossing over the colony	A	A
Single rotor helicopter (ONE H)	Any auditory/ visual disturbance associated with a single rotor helicopter crossing over the colony	A	A
Twin rotor helicopter (TWO H)	Any auditory/ visual disturbance associated with a twin rotor helicopter crossing over the colony	A	A
Jet flare (JET F)	Any disturbance associated with the release of a flare by a jet	A	A
Single rotor helicopter flare (OHEL F)	Any military disturbance associated with the release of a flare by a single rotor helicopter	A	A
Twin rotor helicopter flare (2HEL F)	Any military disturbance associated with the release of a flare by a twin rotor helicopter	A	A
Military Aircraft (MA)	Any auditory/ visual disturbance associated with a fixed wing military aircraft crossing over the colony. Military aircraft are shaped similar to commercial aircrafts.	A	A
Military Aircraft Flare (MAF)	Any disturbance associated with the release of a flare by a fixed wing military aircraft	A	A
Bird call (BC)	Any noise which is associated with a bird call.	N	N
Dog Call (DC)	Although dogs are not allowed at the site, the odd dog bark could be heard from the surrounding fields.	N	N
Bird Aggression (Bird A)	Any aggression initiated by any of the bird species at the site directed at the focal female	N	N
Other Natural	Any novel disturbance events	N	N

*Continued Overleaf

	which has not been accounted for in the descriptions above which had a natural origin.		
Male passes (MPASS)	A male is observed to pass either in front of behind the focal female.	N	AGG
Female passes (FPASS)	A female is observed to pass either in front of behind the focal female.	N	AGG
Pup passes (not own) (P PASS (NO))	A pup, which is not the females own, which is observed to pass either in front of behind the focal female.	N	AGG
Weaned pup pass (WPASS)	A weaned pup is observed to pass either in front of behind the focal female.	N	AGG
Male-Male Aggression (MMAGG)	Any fights which are either observed or heard between two or more male seals. This includes any threat behaviour	N	AGG
Female-Female aggression (FFAGG)	Any fights which are either observed or heard between two or more female seals. These do not include any fights in which the focal female partakes in. Any threat behaviour is also included under this category	N	AGG
Male-Female aggression (MFAGG)	Any fights which are either observed or heard involving both male and female seals. These do not include any fights in which the focal female partakes in. Any threat behaviour is also included under this category	N	AGG
Pup-Female Aggression (PF AGG)	Any fights which are either observed or heard involving both females and pups. These do not include any fights in which the focal female partakes in. Any threat behaviour is also included under this category	N	AGG

2.6. DISTURBANCE EVENT DATA COLLECTION

Disturbance data was collected whilst focal studies were ongoing in order to answer questions 2-8 referenced in section 1.10.1. Noise amplitude levels were monitored throughout each focal video using a CEM noise level meter (Model: CEM DT-85A, level range: 35-100dB: Accuracy; +/-3.0 dB (Ref 94dB at 1 kHz): Frequency: 31.5Hz to 8 kHz). Background amplitude levels were taken either before or during the focal when there was deemed to be no sort of disturbance taking place. It was vital to get these background readings in order to analyse the increases in amplitude during various disturbance events. Minimum and maximum amplitude levels were measured for every 2 minute interval in each of the focal studies. Maximum amplitude levels were also recorded for any sudden disturbance events when possible. As well as the handheld sound meter, an on-board microphone was also attached to the Panasonic camcorder (Model: HC-V720EB-K), which was used in conjunction with the handheld sound meter to check for an accurate reading. The on-board microphone had to be detached at periods of strong wind and so could not be solely relied upon for data collection. Upon returning from the field site, the data from the on-board microphone was deemed unusable due to the fact that it was largely affected by wind, and masked many of the anthropogenic noise signatures.

The number of people moving into and out of the study area in each two minute interval was recorded; as were the number of children and number of photographers in each of these periods in order to answer question 2a set out in section 1.10.1. Identifying the numbers of these particular subsets of individuals was important as previous studies have suggested that these subsets of individuals elicit a greater disturbance response from certain megafauna species (Cassini *et al.,* 2004). During periods of aircraft flyovers from the military base, the number, type of aircraft, and exact time of flyover were noted in a field notebook. In addition to this, during the thirty minute focal any novel disturbance events were recorded in a field notebook; noting the type, time and where possible, amplitude level of the disturbance event. Daily visitor counts and weekly pup and seal population counts (classified into females, males and pups) were obtained from the wardens at the Lincolnshire Wildlife Trust at the end of the field season.

2.7 PROXIMITY MAPPING

During each of the focals, three proximity maps were constructed at 0 minutes, 15 minutes and at the end of the thirty minute focal denoting the location, density and sex of surrounding conspecifics during the focals were constructed (**Figure 2.7; Table 2.7**). Proximity map data was achieved by recording the number of individuals within a 5 seal body length (bl) radius of the focal female; whilst also recording the body length distance between the female and her pup. Mother-pup distance was recorded chiefly to answer question 7a outlined in section 1.10.1. Surrounding individuals were identified as female, male, pup or weaned pup and their orientation in relation to the observer was noted. The focal female was always located at the centre of the map and her pups location was noted by a P* (**Figure 2.7; Table 2.7**). The 5bl limit was placed on the map due to the results of prior research by Redman *et al.,* (2001) suggesting that grey seal mothers rarely move more than 5bl from their pup (Redman *et al.,* 2001). One body length was assumed to be approximately 2m in length. Distances in body length were estimated in the field by eye, using the focal female's body as a point of reference. If an area within the map was obscured from view this was noted on the map and considerations based on this are discussed further in the discussion.

Table 2.7: A description of the colour and symbol codes used for the proximity mapping (Figure2.7).

Colour Key	
	Count 1 (0 minutes)
	Count 2 (15 minutes)
	Count 3 (30 minutes)
Symbol Key	
	Focal Female. Arrows indicate orientation at each of the counts. The focal female was always at the centre of the proximity map
P*	This symbol indicates the position of the focal females pup.
	This symbol represents any other females within 5bl of the focal female at each of the counts (as the symbol here is green it indicates that this female was present at the first count)
	This symbol indicates the presence of any males in the study area. As the symbol is green, it indicates that this male was within 5bl of the focal female on the first count at 0 minutes.
P	This symbol represents the location of any pups (other than the focal females) within 5bl of the focal female. The red colouration here would indicate that this pup was picked up in the third count at thirty minutes into the focal study.
WP	This symbol indicates any weaned pups which may be present within 5bl of the focal female.
	The two coloured circles surrounding this symbol indicate that this female was in the same position for subsequent counts (15 minutes and 30 minutes). Circles surrounding pup and male symbols also highlight individuals which were in the same position in subsequent counts.

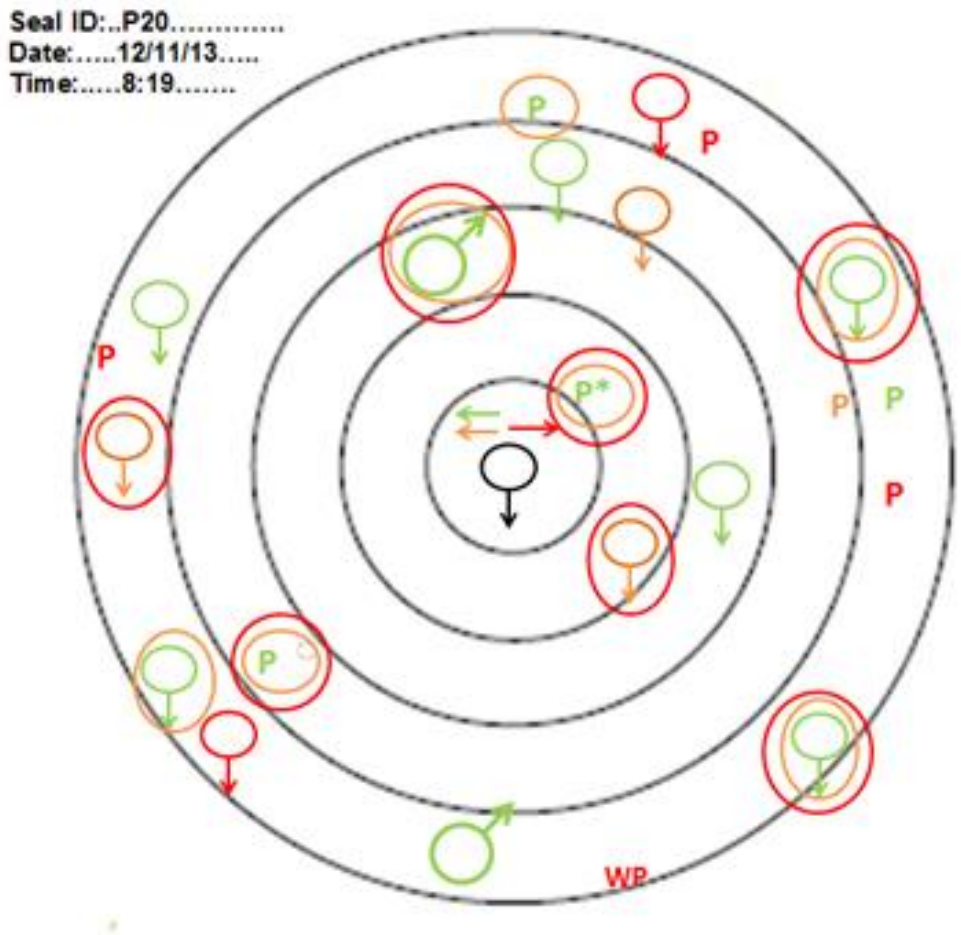

Figure 2.7: An example of a proximity map which was constructed during the field season. Each ring represents one female body length. The female which was the focus of the study can be found in the centre of the proximity map at each count. Measures were made relative to the size of the focal female (given to be around 2m). **Table 2.7** gives details about the coding system used for the proximity mapping. The different colours denote the different timed counts with the green representing 0 minutes, the orange representing fifteen minutes into the study and the red representing thirty minutes into the study.

2.8. WEATHER DATA COLLECTION

The weather data which is used in this study was obtained from the Met Office's British Atmospheric Data Centre (BADC) for the North Somercotes weather station, this being the closest station to the Donna Nook site. Weather data collected included rainfall (mm), wind speed and direction, visibility, relative humidity and ambient temperature (^{o}C). All weather covariates were measured by the Met Office on an hourly basis.

2.9 STATISTICAL ANALYSIS

2.9.1 Colinearity analysis, ICCs and MORQ

The overall aim of the statistical analyses was to identify disturbance parameters which provoke a behavioural response in female grey seals during lactation. There were a number of females which were observed once but could not be re-identified during the study. For the purpose of this study, only females with three or more focal videos were included in the statistical analysis. In total there were 49 females which fit this criterion. For the majority of the analyses, the fine scale behaviours were grouped into broader categories based on previous literature (refer to section 2.5 for the reasoning as to why these behaviours were grouped). These were:

• *Rest*: see **Table 2.5**
• *Comfort Behaviours (CM):* The focal female scratches, shakes or adjusts the position of some part of her body. These also include any drinking and exploration behaviour
• *Movement*: This includes any instances where a female makes a directed movement either toward or away from her pup.
• *Mother-pup Interactions (MPBEH)*: includes both positive and negative physical interactions - play; present; flipper; smell. Nursing is not included in this category.
• *Nursing*: The pup makes contact with the nipple and begins suckling. This broad category includes both alert nursing and rest nursing. Presenting behaviours were not included.
• *Alert*. The female looks around at her environment. This can be a directional look at a disturbance source or a non-directional scan of her surroundings. This does not include an intentional look at her pup (see pup check). This behaviour includes both head-up and head down alert behaviours
• *Pup-check.* See **Table 2.5** (also included pup glance)
• *Vigilance:* This is a combination of the pup check and alert broad categories.
• *Threat.* See **Table 2.5**

Before the behaviours were separated into their broad categories, Colinearity analyses were run on the behavioural parameters in order to reduce the redundancy in the final GLMM models. Colinearity analysis was run using R, and compared all response variables. For each focal the percentage of the total focal time (POF) spent engaged in each fine scale and broad behavioural category was calculated (**Table 2.5**). In addition to this, the frequency of each behaviour was calculated for each focal. Initial univariate analyses on these broad behavioural categories described above focused on

identifying any correlations between the behavioural parameters and any of the confounding response variables which may explain changes in behaviour. Colinearity analysis was achieved by performing Spearman's Rank Correlations using the R programming software. Initial univariate analyses allowed identification of possible response factors which may have provoked a change in the behavioural repertoire of an individual and were therefore deemed important and incorporated into the Generalised linear mixed models (GLMM) which were constructed in order to explain variations in the frequencies/ durations of behaviours between focals and individuals (see section 2.9.2). For the majority of the univariate analysis and later modelling the disturbance categories were split into four broader categories (refer to section 2.6 for the reasoning behind why these disturbance categories were grouped), these being;

- *Aircraft Disturbances*: these involved both military and non-military flyovers and incorporated both fixed wing and helicopter aircraft. Both auditory and visual disturbances by aircraft were included.
- *Pedestrian disturbance*: this incorporated all forms of approaches by visitors on foot, both visual and auditory. Incorporated into this were behaviours such as running, attention behaviour, cameras, mobile phones and the impact of school groups on the seals
- *Natural Disturbances:* These incorporated all forms of biotic natural disturbances that were not directed from conspecifics. These included bird calls, any instances of bird aggression, and dog calls.
- *Aggression by Conspecifics:* This category included any disturbance by conspecifics of any sex. This category not only included aggression by conspecifics but also instances where conspecifics walked across the path of the focal female.

For details regarding which of the fine scale disturbance categorises were incorporated into these four broader disturbances see **Table 2.6.,** where the fourth column separates the behaviours used into Aircraft (Air), pedestrian (P), natural (N) and Aggression (AGG) categories. Again after the initial univariate analyses established which response parameters had an effect on behaviour, colinearity analyses were run on the response parameters, in order to reduce the redundancy in the final GLMM models. Colinearity analyses were run using R, and once again compared all response variables.

2.9.1a Mother-offspring Relationship Quality (MORQ)

In order to answer questions 7a and 7b set out in section 1.10.1 of this study, one needed to see whether disturbance events impacted the MORQ score of female grey seals and their pups. In non-human mammals, particularly primates, mothering styles have often been compared to those observed in humans and techniques used to analyse mother-offspring (MO) relationships in humans have been adapted and developed in order to study non-human animal behaviour (James, 2013). For example Weaver and de Waal, (2002) use Attachment Theory (a study of how secure a human infant feels in the relationship with its mother) in order to compare mother-offspring relationship quality (MORQ) amongst brown capuchins (*Cebu's paella*). By obtaining focal observations of capuchin MO behaviour, the ratio of affiliative to agonistic behaviours between the mother and infant could be calculated, relative to that of all other pairs at the same developmental stage. This study by Weaver and de Waal, (2002) provided a non-invasive, quantitative measure of MORQ for which comparisons could be made amongst the whole study group.

This study wanted to build on the foundations of MORQ study done by James, (2013) on the grey seals at Donna Nook. James, (2013) concentrated on looking at variation of MORQ scores in individuals across lactation stages. This study wanted to take this approach one step further and look at the effects of disturbance on an individual's MORQ score, and compare this in early and late lactation. MORQ calculations are made up from a composite of individual behaviours. MORQ scores take into account several affiliative and rejective behaviours (these are outlined in **Table 2.5**). MORQ is an internal measure of how rejective/affiliative a female is. The measure takes into account not only the amount of time a females spends in affiliative/ rejective behaviours with their pup over the course of a focal but also takes into account the pups age. As the pups age is taken into account this allows comparisons of MORQ scores between females with pups of similar ages. The higher a females MORQ score the more affiliative behaviours the female directs towards her pup.

A MORQ index was calculated for each seal MO pair for each focal video. Although originally designed as a measure of affiliative versus agonistic behaviour. Prior studies along with initial observations of mother-pup interactions in grey seals found that interactions were rarely agonistic and more frequently rejective (James, 2013). For this reason, in this study the MORQ index refers to the ratio of positive mother-pup

interaction to rejective behaviours (Weaver and de Waal, 2002; James, 2013). This MORQ value is then compared with the score obtained for all other females which took part in the study. Table 2.5 categorises behaviours used in the MORQ index into affiliative (A), rejective (R). Those behaviours left blank in the MORQ column were not used in this calculation. “Rejective” behaviours were selected on the basis of the time the mother chose to spend away from her pup, engaging in solitary behaviours, or interacting with conspecifics rather than attending her pup.

Although a female’s main priority on land is to give birth, raise their pup to independence and then mate; seals spend most of their time on breeding colonies engaged in resting behaviours which involve no interaction with their own pup or other conspecifics on the colony. In addition to resting behaviours, females perform a multitude of other behaviours such as interacting with other members of the colony, as well as performing solitary behaviours such as drinking and exploring the habitat, which leaves the pup alone and possibly vulnerable to attack. Due to the fact that these behaviours can leave the pup open to attack from conspecifics, and also may increase the chance of permanent separation of the pup from its mother, all these behaviours are classified as rejective behaviours in terms of the MORQ index. Locomotion and alert behaviours were not included in the MORQ index as it was not always clear whether these behaviours were centred on pup protection and interaction or not. One MORQ index value was calculated from each focal video. The MORQ index provided an indication of those mothers who frequently spent time socialising with their pup, and those who spend little time interacting with their pup; instead focussing their time away from their pup on solitary behaviours. These MORQ values were compared using GLMM models between disturbed and less-disturbed focals to see whether mothers that showed a higher proportion of affiliative behaviours in less-disturbed conditions also showed a high proportion of affiliative behaviours in more disturbed conditions. GLMM tests were used in order to account for individual ID, as a random factor in the analysis process. For this analysis disturbed focals were those with aircraft disturbances present and those videos which had over 50% natural or pedestrian disturbances. This selection criteria was used due to the fact that there were very few focals with zero aircraft, natural and pedestrian disturbances.

MORQ values were standardised using the following approach from James, (2013). In this study, for each focal the percentage of time (POF) a female spent performing affiliative behaviours and the POF a female spent performing rejective behaviours was calculated. After the POF for affiliative and rejective behaviours was calculated for each

focal, affiliative and rejective POFs were calculated for all other mother-pup pairs, where the pup was of the same lactation stage. The focal mother's affiliative value was then divided by the average affiliative value for all of the other mothers in the study, and her rejective value divided by the average rejective value of all the other females in this study. The result of this calculation gave affiliation and rejection values for the focal female relative to all other mothers in the sample (all the females which took part in the study, n=47) (James, 2013). Finally, the relative affiliative value was divided by the relative rejective value in order to give a ratio of affiliation to rejection, relative to all other females in the study which had a pup at the same lactation stage as the focal females pup (James, 2013).

The equation used to calculate the MORQ index for each focal was:
EQUATION 1:

$$\frac{(FA/CA)}{(FR/CR)}$$

Where:
FA = POF spent on affiliation behaviours in focal video by the female
CA = average POF spent on affiliation behaviours by all other females in the study with pups of the same age
FR = POF spent on rejection behaviours in the focal by the focal female
CR = average POF spent on rejection behaviours by other females in the study with pups of the same stage

2.9.1b. Calculating Intra Class Correlations (ICCs)

Calculating the repeatability of an individual's behavioural response to a particular disturbance event was essential in order to answer question 6 set out in section 1.10.1. Repeatability measures were used to test whether a female's behaviour was consistent both across lactation stages and across disturbance contexts. All nine broad behavioural measures (see section 2.9.1. for details on behavioural categories), were tested for repeatability across disturbed and non-disturbed contexts throughout the lactation period. All repeatability estimates were made using the statistical software SPSS, version 19. Though a number of repeatability measures have been used to identify CIDs in behavioural traits. The ICC is good measure repeatability as it identifies both inter- and intra-individual variation, producing a ratio of variation within a population to an individual's phenotypic variance. From this ratio, the presence of CIDs

is confirmed if phenotypic variation is low and population variation is higher (Hayes and Jenkins, 1997). The ICC compared measures from all the female in early lactation and late lactation, as well as incorporating both highly disturbed and minimally disturbed conditions in order to test whether CIDs are robust across situations. In addition an ICC was performed on MORQ values for each female in order to determine whether MORQ scores for individuals were consistent across pup stages and across disturbance contexts.

2.9.2. Assessing the impact that anthropogenic disturbances have on individual behaviour

Assessing the effect that anthropogenic disturbances had on individual behaviour was important to discern in order to address questions 2-8 outlined in section 1.10.1. Initial assessment of the effects that disturbance stimuli have on the behavioural repertoire of female grey seals was conducted using all video focals. The primary aim was to determine if any sources of anthropogenic disturbance provoke a behavioural response in female grey seals during the lactation period. To examine the factors which contribute to variation in individual behavioural responses to disturbance events, generalised linear mixed models (GLMMs) were employed. In the GLMMs, female ID was included as a random factor to account for unequal sampling across females and pseudo-replication (Fossette *et al.,* 2007; Bolker *et al.,* 2009). All models were built using the lme4 package (Bates *et al.,* 2011), in the statistical software R, version 2.13.2 (R Development Core Team, 2011). Final selection of GLMM output followed Richard' (2008) guidelines for AIC selection. This method was chosen due to its selection of a range of models based on a confidence set rather than selection of a single "best" model.

GLMMs were used to identify the effect that location may have on maternal behaviour; with a comparison being made between those females that chose to give birth close to the RAF base and those that chose to give birth next to the public car park. It must be made clear at this point that both sites are still within the part of the colony where visitors can access. This comparison between the two study points was made to test the hypothesis that those females which give birth next to the public car park may be expected to receive more public activity than those which gave birth closer to the MOD base (though still located within the public site boundary). The daily presence of human visitors formed regular noise and visual disturbance at both of these sites. In addition to

this regular fly-overs at the breeding colony provided an additional disturbance source. Although the visual disturbance was concentrated towards the RAF end of the public site, the auditory component of the aerial disturbances could be detected throughout the public site. The GLMM tests were carried out using R statistical programming software. GLMMs were used for this analyses as it allowed the inclusion of both fixed (location) and random (ID) factors to be included in the model. The GLMMs used for this part of the analyses used a binomial family group due to the fact the distribution of the response variable was binomial.

2.9.2a. GLMM analysis at the level of the focal video

Initial GLMM analysis was focused at the whole focal video (30mins) level. The first batch of GLMMs tested whether the duration of time (POF) that the females spent in each of the broad behavioural categories (see 2.9.1) varied in relation to any of the broad disturbance categories (see 2.9.1). Fixed effects in the models included the percentage of time each of the four disturbance categories was present in the focal, as well as other covariates and factors such as; weather parameters (rain, temperature and visibility); the total number of individuals in 5bl (TI5BL); the number of visitors within a 10m radius of the individuals; the maximum and minimum amplitude levels over the course of the focal; how far individuals were from the fence line; whether individuals were at the RAF or public car park side of the public site; the MORQ index for each focal, and the total daily visitor numbers. Day in field and pup stage (as a covariate) were incorporated into the GLMM as an interacting term due to the fact that the two factors are intrinsically linked due to the fact that as the season progressed, the average age of the pups inevitably increases. Individual ID was incorporated as a random factor for the reasons outlined above. For those response behaviours which had a normal distribution or could be transformed to fit a normal distribution the Gaussian family function was adopted (Alert, Comfort, Pup check, Vigilance and Rest) . For those behaviours which could not be transformed to fit a normal distribution (Threat, MPBEH, Nursing and Movement), the binomial family function was adopted due to the fact that the behavioural values were proportional, the data were zero-inflated and the distribution of the behaviours followed that of a binomial function.

In addition to the POF spent in each of the behavioural categories in the focal, the study also analysed whether the frequency of behaviours altered with any of the disturbance parameters using GLMMs. These GLMMs contained the same fixed and

random effects used in the POF GLMM models. Frequencies of behaviours in a focal were extracted by rearranging the data into scan samples using R programming software. This was done by constructing a code which went through the analysis of each focal and selected the behaviour which occurred at a select time period. In this study the time period was ten seconds and so the code allowed for the behaviour at each ten second period to be reported. This alternative approach was used to compare whether adopting a scan or focal approach affected whether a disturbance effect was noted in any of the behavioural parameters. The data for each focal was converted into a series of 10 second scans, where the behaviour at each ten second interval of the focal was noted. The corresponding environmental and anthropogenic conditions which also occurred at that time interval were also extracted. As the focals were of slightly different durations, in order to standardise the frequencies of scans recording each behaviour per focal, instead of directly using the frequencies of scans recording each behaviour, the proportion of each scans in each behavioural category was derived using equation 2. The corresponding GLMMs had a binomial family function due to the fact that the data were proportional.

Equation 2: $$\frac{number\ of\ times\ behaviour\ 'x'\ was\ noted\ in\ a\ focal}{Total\ number\ of\ scans\ in\ a\ focal}$$

2.9.2b. Analysis of the immediate effects of disturbance events

After initial GLMMs concentrated at the level of the focal video was completed, two approaches were used to test the immediate effects that disturbances have on an individual's behaviour. Construction of this analysis was important in order to address question 8 set out in section 1.10.1. All other analyses constructed in this study chiefly focus on the level of the focal video; however previous studies have alluded to the fact that the majority of the behavioural responses elicited by individuals to disturbances events diminish within the first few minutes after the disturbance source has passed, therefore it is essential that this study takes notes of the effects of disturbance on this time scale (Tracey and Flemming, 2007).

The first approach to analysing the immediate responses of individuals to disturbance events involved using GLMMs similar to the approach described in 2.9.2a. In this approach a script in R was constructed which simply takes the behaviour at one time point and asks what the conditions were like (disturbance, neighbour density etc.) at

some pre-defined time before that behaviour; in this case 10 seconds prior. This script allows for a comparison of a behaviour which occurred 10 seconds after a disturbance event. Creating this script allowed for a comparison of the behaviours immediately before and immediately after a disturbance event. In addition to this, the script allowed the study to identify how long the female spent in certain behaviours before and after certain disturbance events. The difference between the GLMMs constructed for this analysis and those conducted in 2.9.2a. was that instead of asking the question 'are alerts more common in a focal when a disturbance happens at that exact time point'; the models now ask 'are alerts more common in a focal when a disturbance occurred 10 seconds prior to that behavioural action'. Ten seconds was chosen to be a suitable time lag due to the fact that, other than resting behaviour, most of the behaviours associated with disturbance events are short in duration. When histograms of the duration of alert, pup check, MPBEH, and threatening behaviours were constructed, the median duration fell below 10 seconds in all categories. The fixed and random effects and model structure incorporated into the GLMM models used for this stage of the analysis were the same as those used at the focal video level described in section 2.9.2a. Analyses were run for all broad behavioural categories outlined in section 2.9.1. ID was kept in the model as a random factor. The GLMMs used a binomial family group due to the binomial distribution of the data used in the analyses.

The second way in which the immediate effects of disturbances was analysed was by analysing the behaviours which occurred two minutes prior to a disturbance event and the behaviours which occurred two minutes after a disturbance event. The durations that females spent in these behaviours was also noted. In order to do this a script was created in R which allowed for the determination of a disturbance event. For this R script a unique ID number for each focal was created, then for each focal, the records where a particular disturbance occurs were selected. The script then examines the original full focal and then extracts and outputs what happens (behaviourally) two minutes before and the two minutes after the disturbance time point. Two minutes was deemed a suitable duration due to the fact that prior studies have alluded to the fact that the behavioural responses of animals to disturbance stimuli are often short lived and last only for a couple of minutes (Duncan *et al.,* 2002; Tracey and Fleming, 2007; Van Polanen Petel *et al*., 2008). In addition to this; other than nursing and resting behaviours, the median durations of all other behaviours were less than two minutes. Mann-Whitney U tests were then used to compare the duration and frequencies of behaviour in the two minutes prior to a particular disturbance event, compared to the

two minutes after the disturbance event. Mann-Whitney U tests were adopted due to the heavily skewed distribution of the data.

2.9.2c. Accounting for pup gender

This analysis was constructed in order to address question 5 set out in section 1.10.1. Simple GLMMs were constructed to identify statistically significant effect that pup sex may have had on any of the behavioural parameters looked at in this study. GLMM models allowed the analyses to take into account both fixed (pup sex) and random (ID) factors. The GLMM models used a binomial family group due to the binomial distribution of the data. Test significance is determined by the inclusion or exclusion of factors from the best fit model. Pup sex was not included as a parameter in the main GLMMs (sections 2.9.2a-2.9.2b), due to the presence of females with pups of unknown sex. If pup sex was included as a factor in the main GLMM models constraints would have been placed on the sample size for these main GLMMs.

2.9.3 Storm Surge on the 5th December 2013

The storm surge on December 5th 2013 caused a sudden and unexpected disturbance to the colony across the Donna Nook colony as well as much of the East coast of England. As the tidal surge was much later in the season than the tidal surge experienced in November 2011, many of the pups had already reached independence and were leaving the colony for the sea (personal observation). However a number of mother-pup pairs were still present at the colony and many of these pairs were separated; in some cases permanently; and a number of pups were seen without a mother in the days following the tidal surge. The day before the tidal surge hit the wardens cut down the fence line allowing the seals to retreat to the surrounding higher ground. Unlike the 2011 tidal surge, only a handful of pups died as a result of the tidal surge and the majority of abandoned pups were taken in by Mablethorpe seal sanctuary. Prior to the tidal surge occurring six females in the study were still on the colony. After the tidal surge hit only four of these females could be located, and one did not have their pup. The new placement of these females made them impossible to film and so the decision was made to stop the field season slightly earlier than planned.

3. RESULTS

This analysis section assesses the effect of individual variation on maternal behaviour, and identifies any evidence that supports the hypothesis that disturbance events may affect female activity budgets during lactation. The analysis section primarily focuses on the estimates of time budgets for both fine and broad scale maternal behaviours (defined in section 2.9.1). The time budgets estimates are calculated from the total percentage of time in a focal video (POF) that females spent performing each of the behaviours. The majority of the focal analysis concentrated on the broad behavioural categories described in section 2.9.1. These broad behavioural categories are; active movement (MOVE), comfort movement (COM), Threat (THREAT), nursing (NURSING), resting (REST), mother-pup interactions both positive and negative, but without nursing (MPBEH), and vigilance (both split into alert (ALERT) and pup-check (PUPC) and combined into one vigilance parameter (VIG)) behaviours. The focal videos were gathered at random and aimed to investigate the general behaviour of an adult female grey seal throughout the lactation period. The study took place at two locations at the public site (see section 2.1).

The first section of this analysis chapter contains preliminary analysis, which will focus on exploring the possible major differences between key parameter groupings (e.g. Early/ late lactation, male/female pups, car park/ RAF site, CIDs). These preliminary analyses are there to guide the final construction of the GLMM models. The second part of this analysis section will explore, through the use of GLMM models, the key factors which influence maternal behaviour and will provide evidence in support or rejection of the question as to whether disturbance parameters appear to affect maternal time budgets in a 30 minute focal. The final part of this analysis chapter will look at the possible immediate effects of disturbance events on maternal behaviour.

3.1 EXPLORATORY ANALYSIS

3.1.1 General patterns of maternal behaviour

Females on breeding colonies spend a vast majority of time resting. The breeding colony at Donna Nook is no exception. During this study, females were observed to spend an average of 63.5% of their time resting during videos (see **Figure 3.2**). The remainder of their time is often spent performing solitary behaviours (e.g. comfort movements), moving around the colony, interacting with conspecifics (aggressively and

towards late lactation, sexually; **Figure 3.2**), performing vigilance behaviours, and interacting with, and nursing their pups.

Excluding nursing and pup-checking behaviours, the percentage of time that females spent interacting with pups (MPBEH) averaged 3.6% of focal videos. These mother-pup interactions were generally of short duration in focal videos and time given to these interaction behaviours was highly variable between mothers. For example, the highest POF spent in mother pup interactions was recorded for mother 37, who spent 50% of one nursing focal interacting with her pup. Some mothers were consistently recorded as spending 0% of focal videos interacting with her pup; for example, mother 1, for whom four random focal videos were recorded as having no mother-pup interactions. The wide variation in the POF females spent in MPBEH may be due to the fact that these behaviours occurred infrequently throughout the day. Females spent around 1.1% of their time engaged in movement behaviours, with POF ranging from 0% to 14.4%. A large amount of variation can be seen also in the POF time females engaged in comfort movements; with a range of engagement in this behaviour observed from 0% up to 68.8%, with an average POF observed at 6.2%.

Vigilance behaviours were of particular interest during this project, having been identified as a key indicator of a reaction to a disturbance event (Cassini *et al*., 2004; Anderson *et al*., 2011 Twiss *et al*., 2012). Vigilance behaviours accounted for 20.1% of all behaviours, with alert behaviours accounting for an average 16.5% and pup-checks an average of 3.6% of the video length. The POF individuals spent performing alerts and pup-checks varied between focal videos. For alert behaviours, POF of each individual focal showed a range of 0% to 89.5%; for pup-checks the range was 0% to just over 40% of each individual focal. The box-plots in **Figure 3.2** show the median POF spent on these broad behavioural categories, and the variation of these across all mothers with focal videos. Results have been split between early and late lactation.

Mother-pup distance was a significant factor affecting the MORQ index values of a female (**Table 3.1**). The Mother-Offspring Relationship Quality (MORQ) index was adapted from that used by Weaver and de Waal, (2002). More affiliative mothers, i.e. those females with a high MORQ score, appeared to retain their pups at a closer proximity on a regular basis than those mothers with a lower MORQ score (**Figure 3.1**).

Table 3.1. Outcomes of GLMM comparing the MORQ index values of individuals and the distance between mother and pup. The + sign indicates categorical factors included in the model which are deemed to significantly affect the MORQ score of an individual. The ΔAICc score give the difference between a particular model and the best model (with the best model scoring a 0). Factors in the GLMM were, mother pup-distance and ID (as a random factor).

MORQ VS MPDIST	Behaviour	Mother-Pup Distance	AICc	ΔAICc	Intercept
	MORQ	+	1687.504	0	2.972991

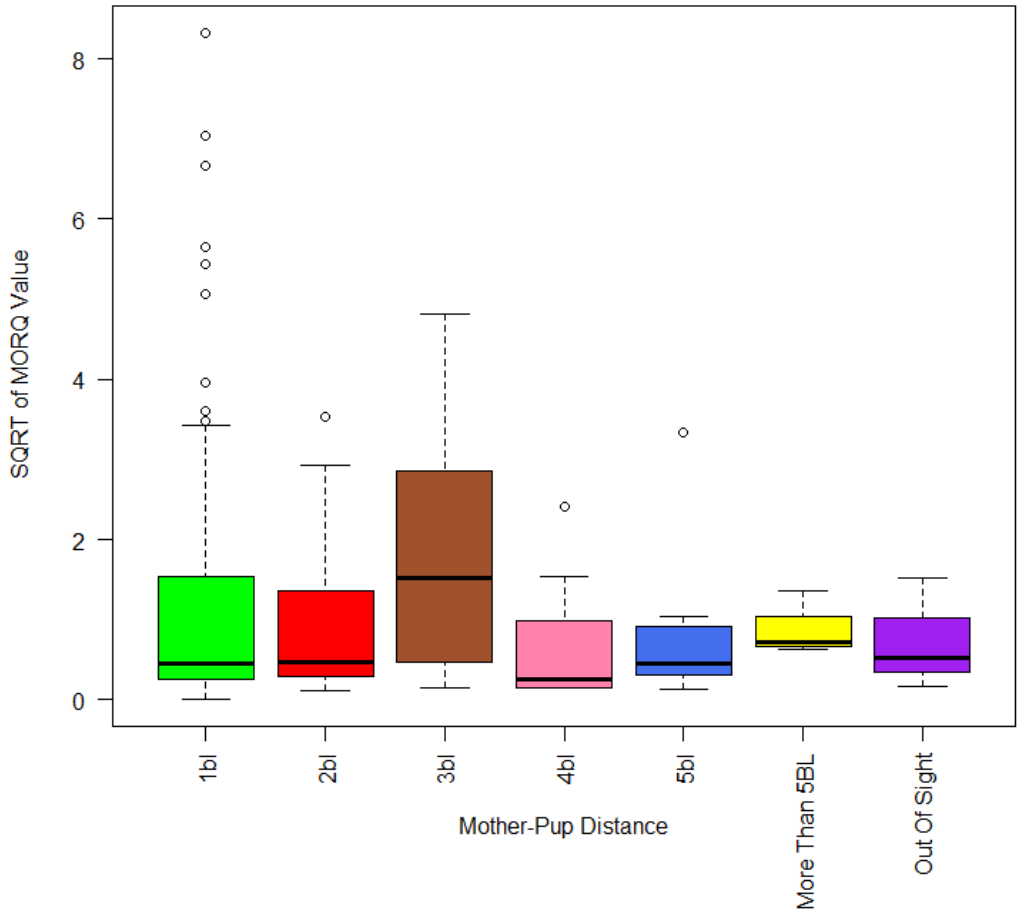

Figure 3.1: The mother-offspring relationship quality (MORQ) Index value (shown as the SQRT of the MORQ index value) of focals compared to the average mother-pup distance observed in that focal. The symbol ○ represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The sqaure root (SQRT) of MORQ values was used insteadof normal MORQ value itself in order to create a sensible graphical representation of the data.

3.1.2. Differences in maternal time budgets across lactation stages, irrespective of disturbance.

The following analyses address the first question set out in section 1.10.1: Does the percentage of the focal (POF) individuals spend on certain maternal behaviours change between early lactation and late lactation irrespective of disturbance events? In this analysis the study compared whether there were changes in the POF spent in the broad behavioural categories outlined in section 2.9.1 between early and late lactation irrespective of whether there was a disturbance in that focal or not.

Mothers spent significantly more time alert and conducting vigilance behaviours overall in early lactation (**Figure 3.2**). Although non-significant, nursing constituted a greater percentage of late lactation videos than those during early lactation. Mother-pup interactions, excluding nursing, accounted for a greater POF during early lactation than observed in late lactation focals. There appeared to be trends for a greater POF spent in comfort movement during late lactation, however, once again the best model for comfort movement did not include lactation stage as a key factor (**Table 3.2**). There is no statistical evidence that lactation stage is a key factor in determining the POF females spent engaged in any of the other behaviours investigated in this study (**Table 3.2**). In focal videos, POF spent on all of the behaviours show high levels of variation about the median (**Figure 3.2)**. This suggests the possibility of variation between individuals in these behaviours, which is further supported by the results of the ICC (see section 3.5.1). Furthermore, preliminary analysis of Mother-offspring relationship quality (MORQ) scores in the grey seals at the Donna Nook site suggested that mothers spent a negligible amount of time acting agonistically towards their own pup (see **Figure 3.2**). Generalised linear mixed models (GLMMs) revealed that lactation stage is an important factor when considering the MORQ scores of a female, with females exhibiting more affiliative behaviours towards pups late in lactation (**Table 3.2.**).

Among the fine scale behavioural categories, maternal time budgets (POF) did not differ between early and late lactation, with the exception that females were more alert, with their heads up early in lactation (**Table 3.3, Figure 3.3**).

Table 3.2: Outcomes of GLMMs comparing the percentage of time during focal videos spent performing broad behaviours between early and late lactation irrespective of disturbance. Throughout this analysis chapter, sample size (N) of a category (e.g. mothers with male pups and female pups, mothers at RAF and car park sites) refers to the number of focal videos used for the study. This sample size number is not equal to the number of females included in that category. This is due to the fact that multiple video focals for each female were taken throughout the study. The number of females in this study is 49. N=249 refers to the number of focals videos. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ΔAICc score give the difference between a particular model and the best model (with the best model scoring a 0). Higher ΔAICc scores indicate models which do a worse job of fitting the data. A ΔAICc score limit of 6 was placed in accordance with Richard, 2008. Factors in the GLMM were lactation stage, and ID (as a random factor). "NA" refers to factors which do not significantly affect the POF individuals spend in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. pup-check, alert, MORQ etc.

Early-late lactation comparison	**Behaviour**	Lactation stage	AICc	ΔAICc	Intercept
N=249	PUPC	NA	17.78415	0	-3.29527
		+	19.54645	1.762301	-3.14658
	ALERT	+	53.08713	0	-1.62393
		NA	54.02688	0.93975	-1.4742
	MOVEMENT	NA	10.77174	0	-4.49754
		+	12.7271	1.955365	-4.34705
	COMFORT	NA	33.16325	0	-2.72523
		+	35.17331	2.01006	-2.77231
	REST	NA	108.509	0	0.553496
		+	109.4278	0.918811	0.432465
	THREAT	NA	16.45849	0	-4.03352
		+	18.44084	1.982352	-3.92924
	NURSING	NA	40.08121	0	-3.30499
		+	42.12972	2.04852	-3.31389
	MOTHER-PUP INTERACTIONS	NA	34.44881	0	-3.20106
		+	36.49584	2.047025	-3.18717
	VIGILANCE	+	62.89427	0	-1.38381
		NA	63.44675	0.552484	-1.2223
	MORQ	+	1701.921	0	2.614554
		NA	1701.946	0.024686	2.861468

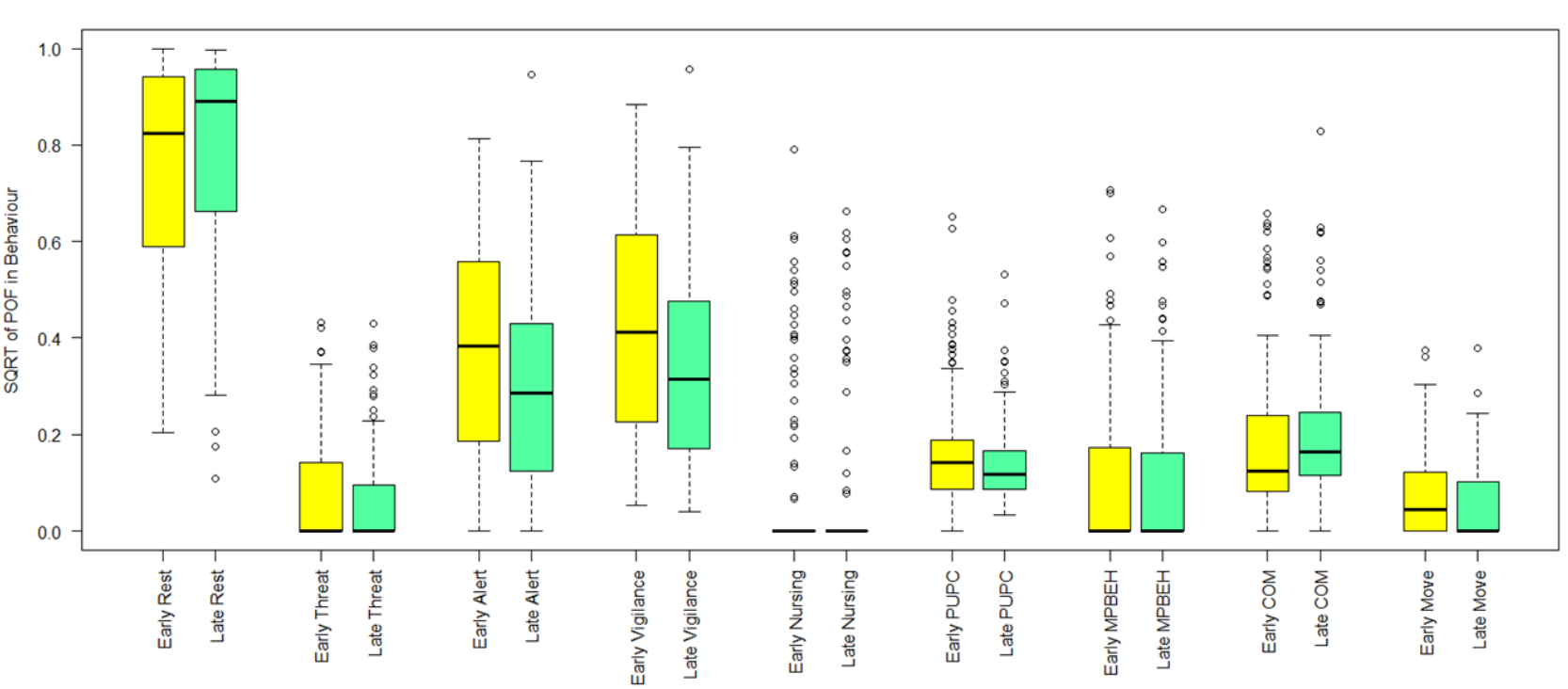

Figure 3.2: The percentage time individuals spent of focal videos (shown as the SQRT of POF) performing the broad scale behaviours analysed in this study. The focals are separated into those taken in early and late lactation for each behavioural category. The symbol ○ represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers.The square root (SQRT) of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data. The vigilance behaviour is a summation of the time spent in alert and pup-check behaviours in the focal.

Table 3.3: Outcomes of GLMM test comparing the percentage of time during focal videos spent performing fine scale behaviours between early and late lactation irrespective of disturbance. The + sign indicates categorical factors which are to be included in the model. "NA" refers to factors which are not included in the model. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ΔAICc score give the difference between a particular model and the best model (with the best model scoring a 0). Higher ΔAICc scores indicate models which do a worse job of fitting the data. A ΔAICc score limit of 6 was placed in accordance with Richard, 2008. Factors in the GLMM were lactation stage, and ID (as a random factor). "NA" refers to factors which do not significantly affect the POF individuals spend in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. alert head-up; alert head-down, pup-glance etc.

Early/ late lactation comparison	Behaviour	Lactation stage	AICc	ΔAICc	Intercept
N. $_{focals}$= 249	Alert head-up	NA	34.59108	0	-2.36223
		+	36.37356	1.782481	-2.26408
	Alert head-down	+	33.22929	0	-2.46201
N. $_{females}$=49		NA	34.43914	1.209846	-2.2875
	Alert nursing	NA	11.84512	0	-5.21096
		+	13.88516	2.040038	-5.28734
	Attempted copulation	NA	4.527755	0	-8.56035
		+	6.522814	1.995059	-7.98841
	Comfort	NA	33.23311	0	-2.76919
		+	35.26166	2.028545	-2.80392
	Copulation	NA	4.079414	0	-11.4062
		+	6.12564	2.046225	-10.8344
	Drink	NA	5.861445	0	-7.11404
		+	7.908144	2.046699	-7.01803
	Exploration	NA	6.505999	0	-6.56342
		+	8.450632	1.944633	-7.21751
	Flipper defence	NA	4.569776	0	-8.23073
		+	6.584141	2.014365	-9.22637
	Movement away from pup(<2 body lengths)	NA	6.955214	0	-5.62112
		+	8.918992	1.963778	-5.3848
	Movement away from pup(>2 body lengths)	NA	5.824976	0.735882	-6.64288
		+	7.874321	0.264118	-6.63353
	Movement towards pup (<2 body lengths)	NA	7.900641	0	-5.2182

*Continued Overleaf

		+	9.914993	2.014352	-5.08593
	Movement towards pup (>2 body lengths)	NA	5.047876	0	-7.24009
		+	7.096571	2.048695	-7.18706
	Mother-pup interactions	NA	9.327226	0	-5.41984
		+	11.30303	1.975804	-5.2171
	Physical aggression	NA	4.363459	0	-8.61013
		+	6.393557	2.030098	-8.18159
	Pup check	NA	16.89847	0	-3.35002
		+	18.70673	1.808264	-3.20963
	Pup glance	NA	7.076948	0	-6.29641
		+	9.063579	1.98663	-6.02098
	PNA	NA	4.177691	0	-9.96496
		+	6.213737	2.036046	-9.39313
	Present	NA	29.61035	0	0.730744
		+	31.60715	1.996802	0.269256
	Rest nursing	NA	35.56946	0	-3.47701
		+	37.61874	2.049282	-3.47377
	Smell	NA	9.087722	0	-5.0512
		+	11.01405	1.926327	-4.83455

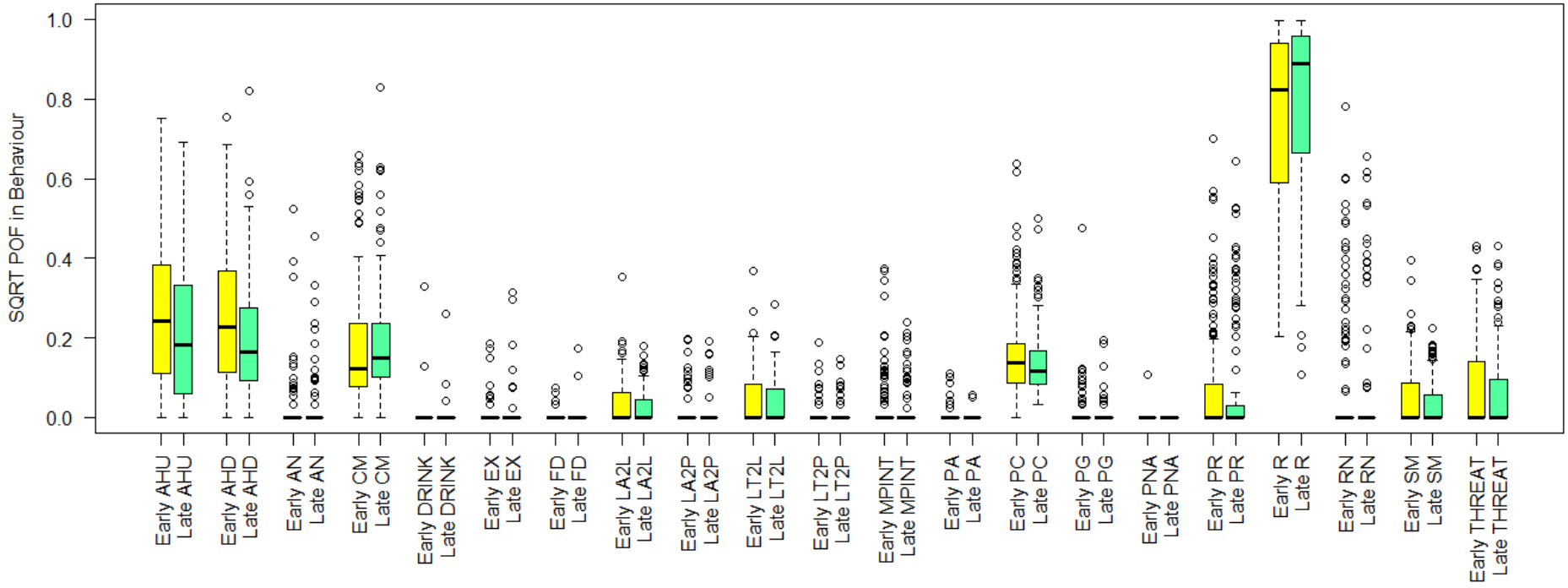

Figure 3.3: percentage time of focal videos (shown as the SQRT of POF) spent performing the fine scale behaviours analysed in this study. The focals are separated into those taken in early and late lactation for each behavioural category. The symbol ○ represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The square-root (SQRT) of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data.

3.1.3 DISTURBANCE EFFECTS ON BEHAVIOUR

3.1.3a The effects of disturbance on the behaviour of individuals

As discussed in section 2.9.1, disturbances were placed into four broad categories, these being: aircraft, pedestrian disturbance, intra-specific aggression, and natural disturbances (section 2.9.1). Generalised linear mixed model (GLMM) analysis revealed that in answer to question 2a and 2b set out in the aims in section 1.10.1; there was no significant difference in the percentage of the focal (POF) individuals spent in any behavioural categories between conditions where aircraft disturbances were present and when they were absent, this is highlighted by the fact the GLMMs revealed that the best model for all of the behavioural categories did not include the occurrence of aircraft disturbances (**Table 3.4; Figure 3.4a**). In answer to question 2a set out in section 1.10.1; when comparisons were made between focals which contained pedestrian disturbances and those which did not, GLMMs revealed that the occurrence of pedestrian disturbance was a key factor in determining the POF females spent engaged in pup-checking behaviours. Females spent significantly more time engaged in pup checking behaviours than in focals where pedestrian disturbances were absent. In addition to this, females spent significantly less time engaged in mother-pup behaviours (MPBEH) when pedestrian disturbances were present in the focal compared to when they were absent (**Table 3.4; Figure 3.4b**.). POF spent engaged in any of the other behavioural categories did not differ between those focals in which pedestrian disturbances were present, and those in which they were absent.

As noted in section 2.9.1, natural disturbances were split into two broad categories, these being; intraspecific aggressive encounters and more general natural disturbances which did not arise from conspecifics. To answer 3 set out in section 1.10.1; this being whether natural disturbances elicited a similar behavioural response to anthropogenic disturbances, when comparing focals where intraspecific aggression was present with those where they were absent, the GLMMs revealed that the occurrence of these aggressive encounters was a factor which significantly affected the POF time that females at the Donna Nook site spent engaged in pup-checking, threat and vigilance behaviours than in focals where intraspecific aggressions did not occur within 20m of the focal female (**Table 3.4; Figure 3.4c**). Furthermore, in those focal in which intraspecific aggressive encounters took place, females spent less time engaging in resting behaviours than when intraspecific encounters did not occur. The POF that females spent engaged in all of the behavioural categories explored in this study did not differ significantly between those focals in which there were natural

disturbances, and those in which they were absent (**Figure 3.4d**). This being the case the overall lack of a behavioural response by individuals to both natural disturbance events is consistent with individual's lack of responses to anthropogenic disturbances. If anything can be noted is that the responses of individuals to natural disturbances are slightly more extreme than their responses to anthropogenic disturbances. Although for this to be fully deciphered the impacts that these responses have on pup survival would need to be established.

With regards to the MORQ scores of females in the presence of the different disturbance stimuli, the GLMMs revealed that MORQ score was not affected by the occurrence of natural, pedestrian or aircraft disturbance stimuli. However, MORQ scores were impacted by intraspecific aggression disturbances, with lower MORQ scores in focals where these disturbances were present when compared to focals in which intraspecific aggressive encounters were absent (**Table 3.4.**).

Table 3.4 Outcomes of GLMM tests comparing the POF spent in behaviours between disturbed and non-disturbed conditions. The type of disturbance is listed in the left hand column. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ∆AICc score give the difference between a particular model and the best model (with the best model scoring a 0). Higher ∆AICc scores indicate models which do a worse job of fitting the data. A ∆AICc score limit of 6 was placed in accordance with Richard, 2008. Factors in the GLMM were disturbance category (e.g. aircraft), and ID (as a random factor). "NA" refers to factors which do not significantly affect the POF individuals spend in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. pup-check, alert, comfort etc.

Aircraft	Behaviour	Occurrence of Aircraft	AICc	∆AICc	Intercept
	PUPC	NA	17.78415	0	-3.29527
		+	19.26398	1.479827	-3.06654
	ALERT	NA	53.08713	0	-1.62391
		+	54.89948	1.812356	-1.54345
	MOVEMENT	NA	10.77174	0	-4.49754
		+	12.81569	2.043955	-4.54297
	COMFORT	NA	33.16325	0	-2.72523
		+	35.10429	1.941036	-2.64209
	REST	NA	108.509	0	0.553516
		+	109.3513	0.842285	0.412361
	THREAT	NA	16.45849	0	-4.03354
		+	18.31729	1.858798	-4.26783
	NURSING	NA	40.08121	0	-3.30499
		+	41.90065	1.819445	-3.15313
	MOTHER-PUP INTERACTIONS	NA	34.44881	0	-3.20103
		+	36.17881	1.729998	-3.03183
	VIGILANCE	NA	62.89427	0	-1.38381
		+	64.30437	1.410101	-1.2625
	MORQ	NA	1701.929	0	3.143633
		+	1701.946	0.016435	2.861468

*Continued Overleaf

PEOPLE	Behaviour	Occurrence of people	AICc	∆AICc	INT
	PUPC	+	17.78415	0	-3.29527
		NA	19.83222	2.048066	-3.26591
	ALERT	NA	53.08713	0	-1.62391
		+	55.06838	1.981249	-1.51983
	MOVEMENT	NA	10.77174	0	-4.49754
		+	12.80973	2.037998	-4.35044
	COMFORT	NA	33.16325	0	-2.72523
		+	34.87356	1.710311	-3.14479
	REST	NA	108.509	0	0.553516
		+	110.3903	1.881371	0.684413
	THREAT	NA	16.45849	0	-4.03354
		+	18.50604	2.047557	-4.08392
	NURSING	NA	40.08121	0	-3.30499
		+	41.25794	1.176733	-4.39254
	MOTHER-PUP INTERACTIONS	+	34.44881	0	-3.20103
		NA	36.48819	2.039381	-3.28136
	VIGILANCE	NA	62.89427	0	-1.38381
		+	64.87657	1.982301	-1.28768
	MORQ	NA	1701.558	0	2.591288
		+	1701.946	0.387482	2.861468
AGG	**Behaviour**	**Occurrence of Aggression**	**AICc**	**∆AICc**	**INT**
	PUPC	+	17.78415	0	-3.29527
		NA	19.72306	1.938913	-3.43711
	ALERT	+	52.62796	0	-1.97943
		NA	53.08713	0.459167	-1.62391
	MOVEMENT	NA	10.77174	0	-4.49754
		+	12.6684	1.896664	-4.81783
	COMFORT	NA	33.16325	0	-2.72523
		+	35.21152	2.048271	-2.71491
	REST	+	107.886	0	0.816515
		NA	108.509	0.622997	0.553516
	THREAT	+	16.45849	0	-4.03354
		NA	17.66732	1.208835	-4.72342
	NURSING	NA	40.08121	0	-3.30499
		+	42.03092	1.949713	-3.18381
	MOTHER-PUP INTERACTIONS	NA	34.44881	0	-3.20103
		+	36.42544	1.976626	-3.30974
	VIGILANCE	+	62.32294	0	-1.71485
		NA	62.89427	0.571327	-1.38381
	MORQ	+	1700.178	0	2.061228
		NA	1701.946	1.767965	2.861468
Natural	**Behaviour**	**Occurrence of Natural Disturbance**	**AICc**	**∆AICc**	**INT**
	PUPC	NA	17.78415	0	-3.29527
		+	19.3064	1.522253	-3.12076
	ALERT	NA	53.08713	0	-1.62391
		+	55.12993	2.042803	-1.61311
	MOVEMENT	NA	10.77174	0	-4.49754

*Continued Overleaf

	+	12.79219	2.020457	-4.58239
COMFORT	NA	33.16325	0	-2.72523
	+	34.02015	0.856901	-2.52448
REST	NA	108.509	0	0.553516
	+	110.3108	1.801839	0.502669
THREAT	NA	16.45849	0	-4.03354
	+	18.45727	1.998784	-3.95228
NURSING	NA	40.08121	0	-3.30499
	+	41.74837	1.667165	-3.48785
MOTHER-PUP INTERACTIONS	NA	34.44881	0	-3.20103
	+	36.37213	1.923322	-3.2967
VIGILANCE	NA	62.89427	0	-1.38381
	+	64.77962	1.885358	-1.3345
MORQ	NA	1701.946	0	2.861468
	+	1702.232	0.285864	2.846198

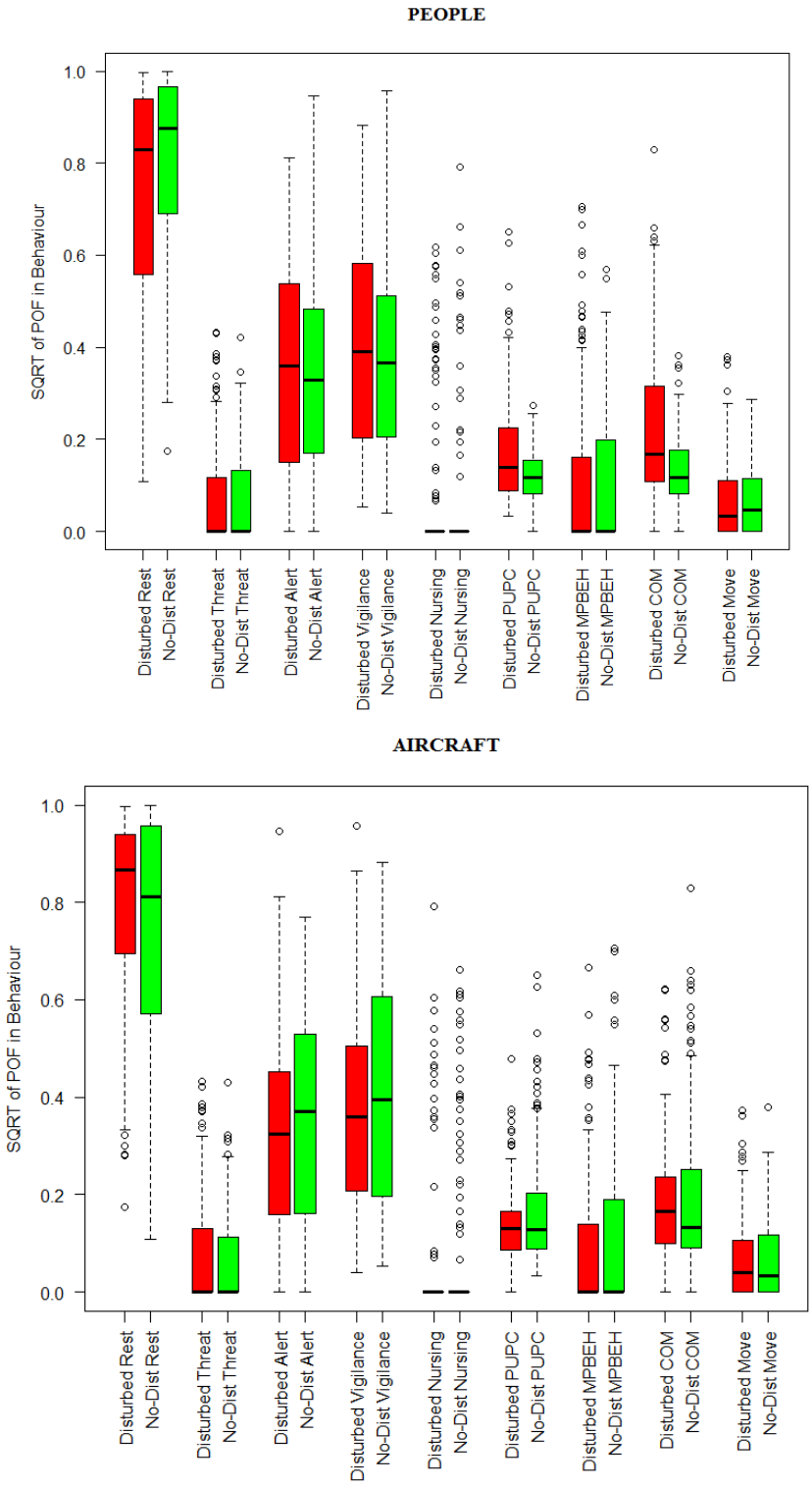

Figure 3.4. a) pedestrian disturbance b) aircraft disturbance. Percentage of the focal (POF) (shown as the SQRT of POF) that an individual spent performing each of the broad category behaviours analysed in this study. The focals are separated into those taken in disturbed and non-disturbed conditions. The symbol O represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The square-root (SQRT) of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data.

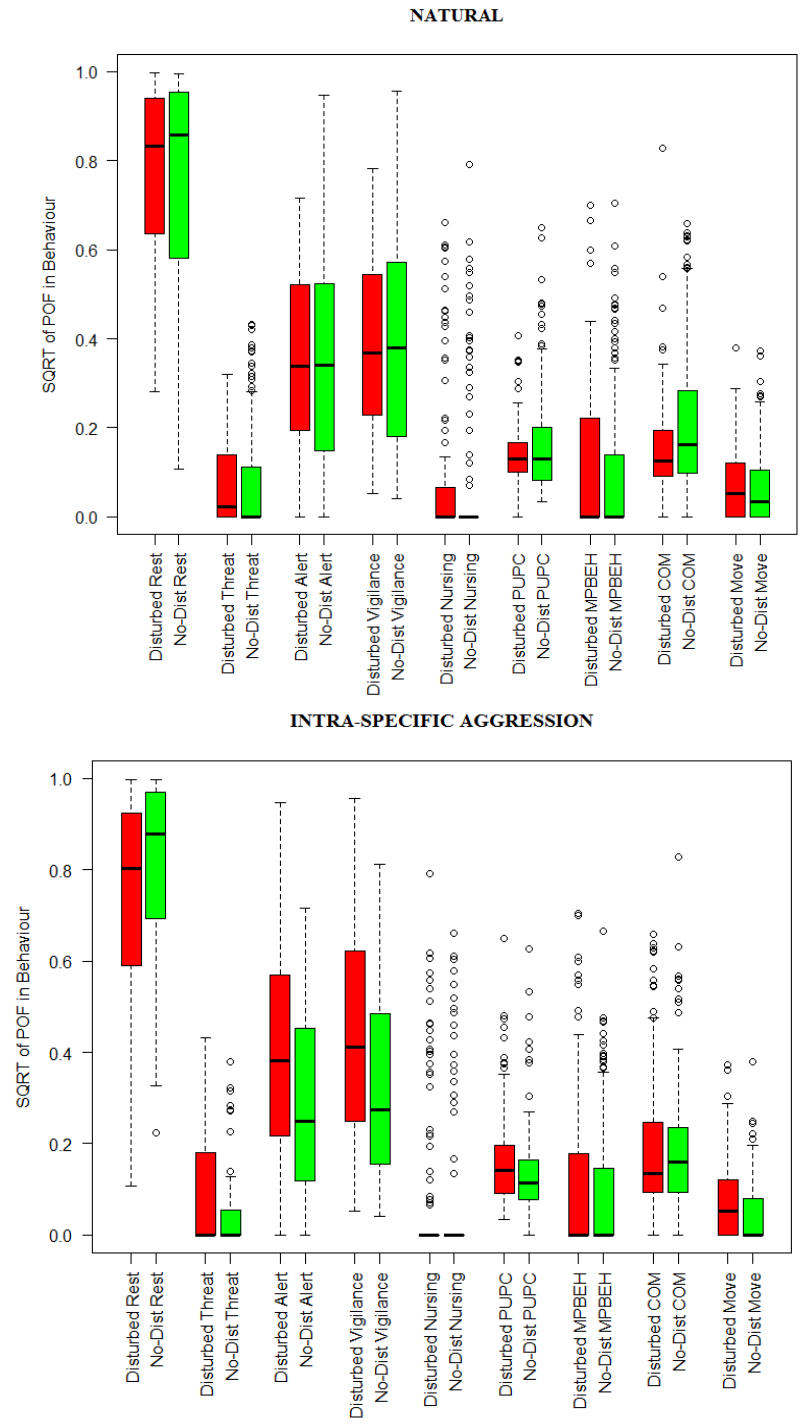

Figure 3.4. c) natural disturbance d) intraspecifc aggression. Percentage of the focal (POF) (shown as the SQRT of POF) that an individual spent performing each of the broad category behaviours analysed in this study. The focals are separated into those taken in disturbed and non-disturbed conditions. The symbol O represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The square-root (SQRT) of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data. The vigilance behaviour is a summation of the time spent in alert and pup-check behaviours in the focal.

3.1.3b. Aircraft Type

The following analyses addresses question 2b set out in section 1.10.1. This being; what types of aerial anthropogenic disturbances have the greatest impact on female behaviour? Aircraft flyovers from military sources were limited to weekdays, often between the hours of 9am and 5pm. These aircraft were low flying and provided both visual and auditory sources of disturbance to the breeding seals. The noise produced from the military aircrafts frequently exceeded 100dB, with jets producing the loudest sounds. Non-military aircraft referred to passenger jets which flew above the colony. These flew at much higher altitudes than the military aircraft and only caused auditory disturbances to the colony. The noise produced from the passenger jets was much quieter than their military counterparts, often measuring in at under 80dB on the sound meter. .

Analysis of the effect that non-military aircraft and one rotor helicopters had on the POF females spent in certain behaviours indicated that there was no significant difference in the average percentage of the focal (POF) individuals spent in any behavioural categories between conditions where these two aircraft disturbances were present in a focal and when they were absent in a focal (**Table 3.5; Figure 3.5c and e**). With respect to focals which contained Jet aircraft, the generalised linear mixed models (GLMMs) constructed for each behaviour, revealed that females spent significantly more time engaging in threat behaviours when jets were present in a focal than in those focals without jets (**Figure 3.5a**). For all other behavioural categories the presence of jets was not a significant factor (**Table 3.5**). In addition to this, females spent significantly more time engaging in pup-checking behaviours in focals without military aeroplanes when compared to focals where military aeroplanes is present (**Figure 3.5b**). Once again all other behavioural categories were insignificantly different between those focals with military aeroplanes and those without. Finally in comparisons of focals with and without twin rotary helicopters, the GLMMs revealed that individuals spent a significantly greater time engaged in comfort behaviours in focals without twin rotary helicopters than in those focals where twin rotary helicopters were present (**Table 3.5; Figure 3.5d**). Again, all other behavioural categories were insignificantly different between those focals which did, and those which did not contain twin rotary helicopters.

In conclusion the results of this analysis suggest that overall the type of aircraft does not significantly affect the behavioural response of females to disturbance. Largely there is very little behavioural response by females to any aircraft disturbance at the

public site. As the type of aircraft disturbance did not have any significant effect on the behavioural response exhibited by females to aircraft disturbances, from this point on all five aircraft disturbances will be labelled under the heading “aircraft disturbances”.

Table 3.5 Outcome of GLMM tests comparing the POF spent in behaviours between focals with specified aerial disturbances and those in which the aerial disturbances were absent. The first column on the left indicates the type of aircraft disturbance being analysed. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ΔAICc score give the difference between a particular model and the best model (with the best model scoring a 0). Higher ΔAICc scores indicate models which do a worse job of fitting the data. A ΔAICc score limit of 6 was placed in accordance with Richard, 2008. Factors in the GLMM was the occurrence of a particular aircraft (e.g. jet), and ID (as a random factor). “NA” refers to factors which do not significantly affect the POF individuals spend in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. pup-check, alert, comfort etc. and for each mode of aircraft disturbance.

JET VS NO JET	**Behaviour**	**Occurrence of a jet**	**AICc**	**ΔAICc**	**Intercept**
	PUPC	NA	17.78415	0	-3.29527
		+	19.7812	1.997053	-3.26376
	ALERT	NA	53.08713	0	-1.62391
		+	54.34307	1.255937	-1.5636
	MOVEMENT	NA	10.77174	0	-4.49754
		+	12.77463	2.002898	-4.44634
	COMFORT	NA	33.16325	0	-2.72523
		+	35.20812	2.044866	-2.71786
	REST	NA	108.509	0	0.553516
		+	110.1787	1.669736	0.519904
	THREAT	+	16.45849	0	-4.03354
		NA	17.82299	1.364501	-4.23922
	NURSING	NA	40.08121	0	-3.30499
		+	42.12756	2.046358	-3.31298
	MOTHER-PUP INTERACTIONS	NA	34.44881	0	-3.20103
		+	36.47919	2.030378	-3.18255
	VIGILANCE	NA	62.89427	0	-1.38381
		+	64.08259	1.188324	-1.32513
	MORQ	NA	1701.071	0	3.007135
		+	1701.946	0.874857	2.861468
military aircraft vs No Military Aircraft	**Behaviour**	**Occurrence of military aircraft**	**AICc**	**ΔAICc**	**INT**
	PUPC	+	17.78415	0	-3.29527
		NA	19.45684	1.672688	-3.23047
	ALERT	NA	53.08713	0	-1.62391
		+	54.95538	1.868254	-1.59919
	MOVEMENT	NA	10.77174	0	-4.49754
		+	12.79812	2.026387	-4.5315
	COMFORT	NA	33.16325	0	-2.72523
		+	35.19224	2.028992	-2.73868
	REST	NA	108.509	0	0.553516
		+	110.1992	1.69022	0.52626
	THREAT	NA	16.45849	0	-4.03354

*Continued Overleaf

		+	18.48426	2.025774	-4.06071
	NURSING	NA	40.08121	0	-3.30499
		+	41.91277	1.831564	-3.25393
	MOTHER-PUP INTERACTIONS	NA	34.44881	0	-3.20103
		+	36.48697	2.038159	-3.18914
	VIGILANCE	NA	62.89427	0	-1.38381
		+	64.5033	1.609034	-1.34841
	MORQ	NA	1700.976	0	2.961859
		+	1701.946	0.970056	2.861468
Non-military aircraft vs no non-military aircraft	**Behaviour**	**Occurrence of non-military aircraft**	**AICc**	**∆AICc**	**INT**
	PUPC	NA	17.78415	0	-3.29527
		+	19.82646	2.042304	-3.28207
	ALERT	NA	53.08713	0	-1.62391
		+	55.04708	1.959949	-1.64811
	MOVEMENT	NA	10.77174	0	-4.49754
		+	12.82088	2.049146	-4.50187
	COMFORT	NA	33.16325	0	-2.72523
		+	34.97528	1.812025	-2.66886
	REST	NA	108.509	0	0.553516
		+	110.4844	1.975442	0.570252
	THREAT	NA	16.45849	0	-4.03354
		+	18.47569	2.017201	-4.07574
	NURSING	NA	40.08121	0	-3.30499
		+	41.8237	1.742497	-3.40168
	MOTHER-PUP INTERACTIONS	NA	34.44881	0	-3.20103
		+	36.49537	2.046562	-3.20917
	VIGILANCE	NA	62.89427	0	-1.38381
		+	64.8863	1.992037	-1.40164
	MORQ	NA	1700.892	0	2.658205
		+	1701.946	1.053627	2.861468
One rotor helicopter vs no one rotary helicopter	**Behaviour**	**Occurrence of one rotor helicopter**	**AICc**	**∆AICc**	**INT**
	PUPC	NA	17.78415	0	-3.29527
		+	19.83314	2.048985	-3.29733
	ALERT	NA	53.08713	0	-1.62391
		+	54.9655	1.878375	-1.6461
	MOVEMENT	NA	10.77174	0	-4.49754
		+	12.7838	2.012067	-4.46447
	COMFORT	NA	33.16325	0	-2.72523
		+	35.18782	2.02457	-2.73819
	REST	NA	108.509	0	0.553516
		+	110.5375	2.028479	0.547736
	THREAT	NA	16.45849	0	-4.03354
		+	18.47187	2.013386	-4.00713

*Continued Overleaf

	NURSING	NA	40.08121	0	-3.30499
		+	41.56196	1.480756	-3.23906
	MOTHER-PUP INTERACTIONS	NA	34.44881	0	-3.20103
		+	36.13875	1.689935	-3.14854
	VIGILANCE	NA	62.89427	0	-1.38381
		+	64.78839	1.894121	-1.40326
	MORQ	NA	1700.392	0	2.738997
Two rotor helicopter vs no two rotor helicopter	**Behaviour**	**Occurrence of two rotor helicopter**	**AICc**	**∆AICc**	**INT**
	PUPC	NA	17.78415	0	-3.29527
		+	19.57347	1.789316	-3.25353
	ALERT	NA	53.08713	0	-1.62391
		+	55.1342	2.04707	-1.62615
	MOVEMENT	NA	10.77174	0	-4.49754
		+	12.79464	2.022904	-4.52619
	COMFORT	NA	33.16325	0	-2.72523
		+	34.53082	1.367571	-2.67449
	REST	NA	108.509	0	0.553516
		+	109.5986	1.089669	0.519473
	THREAT	NA	16.45849	0	-4.03354
		+	18.42689	1.968397	-4.07449
	NURSING	NA	40.08121	0	-3.30499
		+	40.94019	0.858989	-3.23212
	MOTHER-PUP INTERACTIONS	NA	34.44881	0	-3.20103
		+	35.90428	1.455463	-3.14454
	VIGILANCE	NA	62.89427	0	-1.38381
		+	64.91492	2.020658	-1.37658
	MORQ	NA	1699.677	0	3.004396
		+	1701.946	2.268978	2.861468

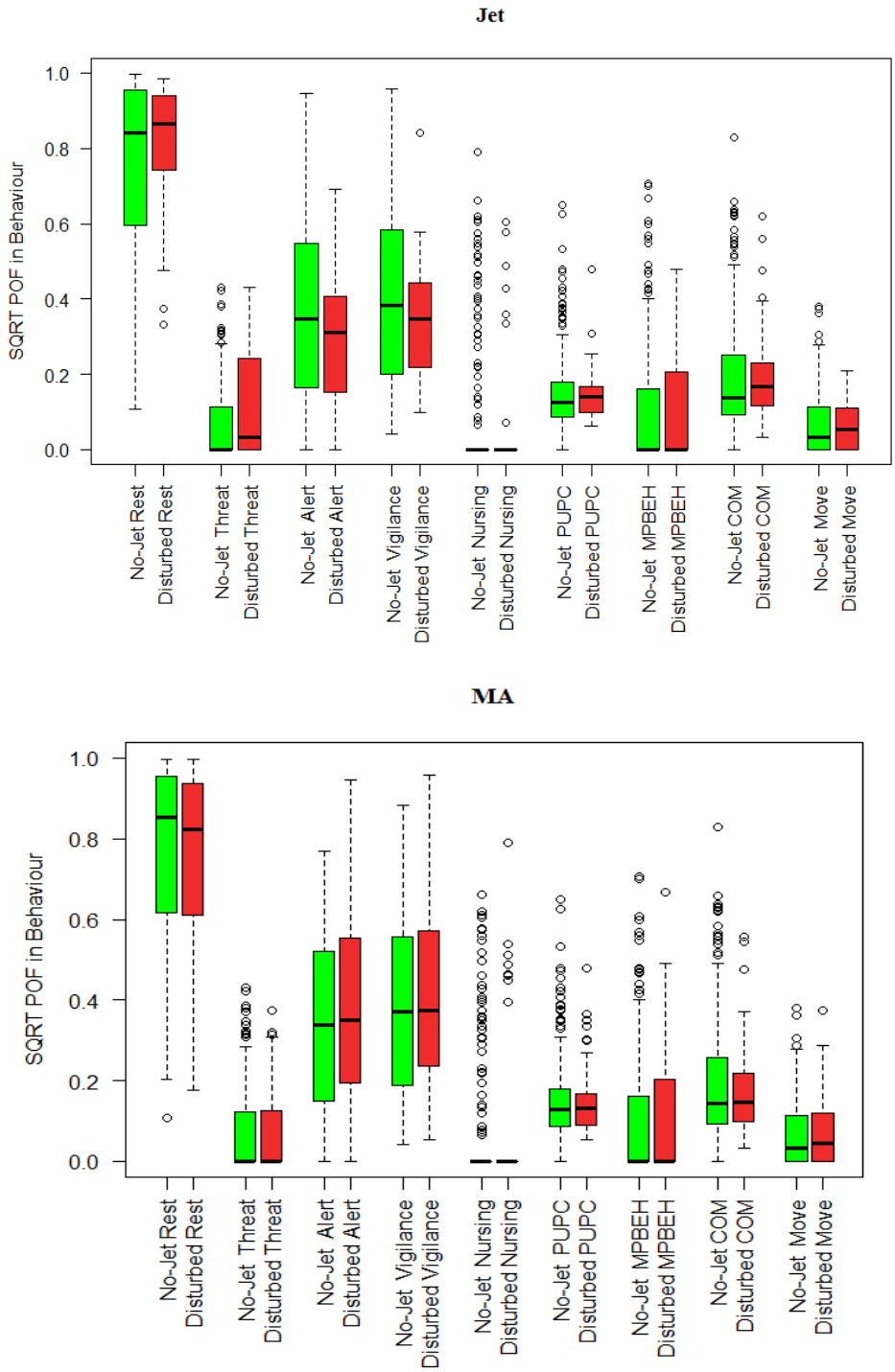

Figure 3.5: a) Jet b) Military Aircraft (MA). Percentage of the focal (POF) (shown as the SQRT of POF) that an individual spent performing each of the broad category behaviours analysed in this study. The focals are separated into those taken in the presence and absence of each aircraft type. The symbol ○ represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The square-root (SQRT) of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data. The vigilance behaviour is a summation of the time spent in alert and pup-check behaviours in the focal.

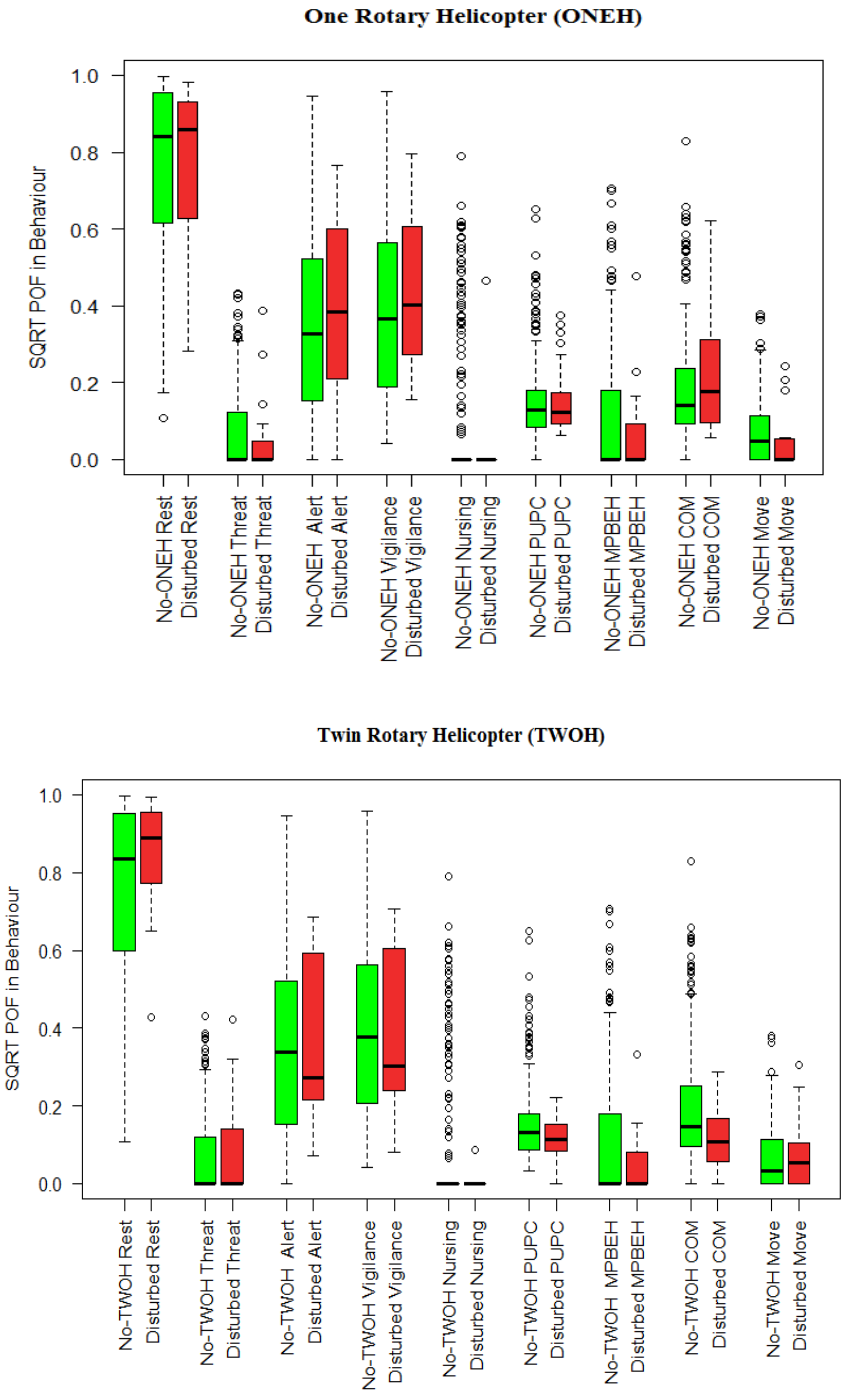

Figure 3.5: C) one Rotary Helicopter (ONEH) D) Twin Rotary Helicopter (TWOH). Percentage of the focal (POF) (shown as the SQRT of POF) that an individual spent performing each of the broad category behaviours analysed in this study. The focals are separated into those taken in the presence and absence of each aircraft type. The symbol ○ represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The square-root (SQRT) of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data. The vigilance behaviour is a summation of the time spent in alert and pup-check behaviours in the focal.

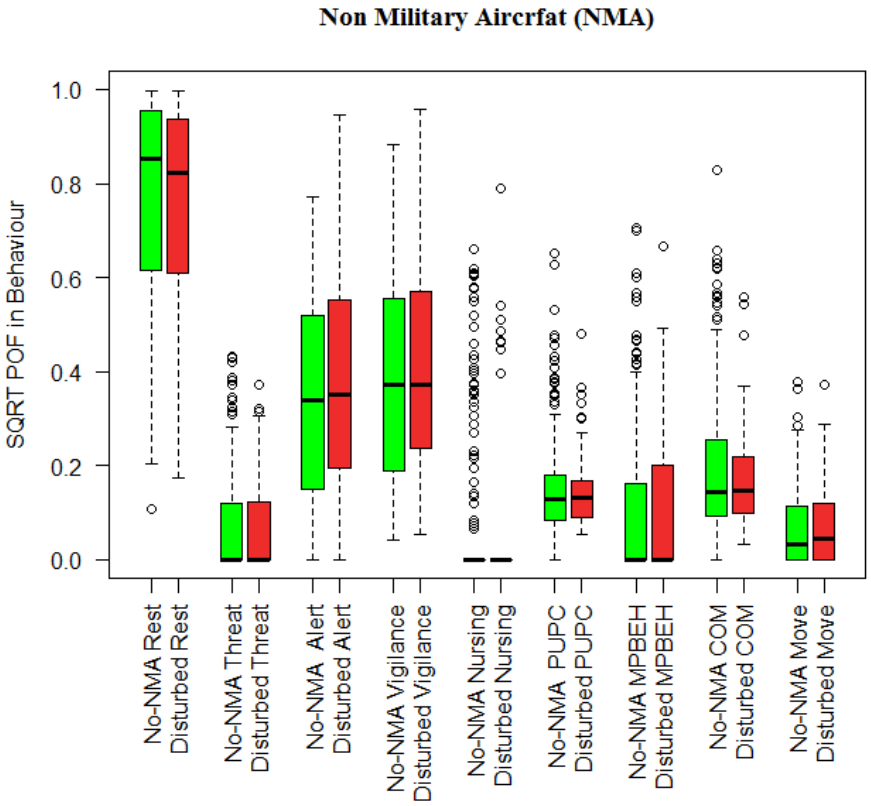

Figure 3.5) Non-Military Aircrfat (NMA). Percentage of the focal (POF) (shown as the SQRT of POF) that an individual spent performing each of the broad category behaviours analysed in this study. The focals are separated into those taken in the presence and absence of each aircraft type. The symbol ○ represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The square-root (SQRT) of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data. The vigilance behaviour is a summation of the time spent in alert and pup-check behaviours in the focal.

3.1.3c The effects of sound and visitor attendance parameters on behaviour.

The following analyses addresses question 2a set out in section 1.10.1. This being; Do anthropogenic disturbance events affect female grey seal breeding behaviour? Females at the colony were exposed to around twelve hours of noise and visual disturbances from visitors to the site each and every day while the study took place. Noise and movement from visitors remaining behind the barrier was constant from dawn up to, and often exceeding dusk. Although visitors to the public site were mostly kept behind a fence line and away from the colony, occasionally at the weekend photographers were allowed to move beyond this barrier, to visit the outer colony located at the tide line, roughly 500m from the main inland colony. The photographer's access point onto the colony was next to the public car park.

In answer to question 2a outlined in section 1.10.1., regarding whether anthropogenic disturbances affected the behaviour of breeding female grey seals, the generalised linear mixed models (GLMMs) revealed that neither the average number of people within 10m of the focal female, the average number of children within 10m of the focal female, nor any of the sound parameters investigated (background sound level, average minimum amplitude per focal and average maximum sound level had an effect on any of the behavioural parameters investigated in this study (**Table 3.6**).

In contrast, the total daily visitor numbers had significant effects on the percentage of the focal (POF) females spent engaged in alert and vigilance behaviours at Donna Nook. Alert and vigilance levels of female grey seals on the colony were higher when the number of visitor numbers was at its lowest. As the numbers of visitors increased, the levels of engagement in alert and vigilance behaviours in the focals fell (**Table 3.6**). None of the other behavioural categories were correlated with the number of people visiting the attraction during the day. In addition to the effects that the number of daily visitors to the colony had on behaviour, the number of photographers present within a 10m radius of the female at the fence line affected the behaviour of females. The GLMMs revealed that females were significantly more likely to engage in more threatening behaviours when the number of photographers within a 10m radius of the fence line was higher (**Table 3.6**). Again, the number of photographers was not seen to be a significant factor affecting any of the other behavioural categories.

Finally, the results of the GLMMs found that the POF females spent engaged in comfort, resting, pup checking and vigilance behaviour was significantly affected by the distance that the female chose to breed in relation to the fence line. Those females which chose to pup closer to the fence line had significantly higher levels of comfort

movements, pup checking and vigilance behaviours than those females which chose to pup further from the fence (**Table 3.6**). In addition to this, females which pupped closer to the fence also spent significantly less time engaging in resting behaviours than those females which settled further from the fence line **(Table 3.6**). Once again the distance from the fence line was not a significant factor which affected the POF females spent engaged in any of the other behavioural categories.

Table 3.6: Outcomes of GLMM tests analysing whether the level of auditory disturbance and visitor attendance parameters affected the percentage of the focal (POF) females spent engaged in a certain behaviour. The behaviour being analysed can be found in the left hand column. The ΔAICc score give the difference between a particular model and the best model (with the best model scoring a 0). Higher ΔAICc scores indicate models which do a worse job of fitting the data. A ΔAICc score limit of 6 was placed in accordance with Richard, 2008. Factors in the GLMM was the occurrence of a particular disturbance (e.g. Distance to fence, daily visitor numbers etc.), and ID (as a random factor). "NA" refers to factors which do not significantly affect the POF individuals spend in certain behaviours according to the model. Negative numbers indicate a negative correlation. A GLMM was run for each behaviour category e.g. pup-check, alert, comfort etc. and for each disturbance source.

Behaviour	Disturbance	Occurrence of disturbance	AICc	ΔAICc	INT
Alert	Distance to fence	NA	53.08713	0	-1.62393
		-0.0136	54.15345	1.066321	-1.35696
	No. daily visitors	-4.14E-05	53.08713	0	-1.62391
		NA	55.06928	1.98215	-1.57835
	No. People in 10m radius	NA	53.08713	0	-1.62391
		0.001323	55.1137	2.026568	-1.64982
	No. Photographers in 10m	NA	53.08713	0	-1.62391
		0.024594	55.04623	1.959099	-1.67052
	No. children in 10m	NA	53.08713	0	-1.62391
		0.027124	54.7779	1.690767	-1.67766
	background sound (dB)	NA	53.08713	0	-1.62391
		-0.01516	54.98997	1.902843	-0.85165
	Minimum sound (dB)	NA	53.08713	0	-1.62391
		0.012023	54.98655	1.899419	-2.26906
	Maximum sound (dB)	NA	53.08713	0	-1.62391
		-0.00889	54.5803	1.493173	-0.88031
COMFORT	Distance to fence	-0.03646	32.71875	0	-2.06966
		NA	33.16325	0.444501	-2.72525
	No. daily visitors	NA	33.16325	0	-2.72523
		3.22E-05	35.1945	2.031247	-2.76179
	No. People in 10m radius	NA	33.16325	0	-2.72523
		0.000605	35.21064	2.047389	-2.73706
	No. Photographers in 10m	-0.07304	33.16325	0	-2.72523
		NA	34.93975	1.776503	-2.59859
	No. children in 10m	NA	33.16325	0	-2.72523
		-0.00257	35.21154	2.048287	-2.72046

*Continued Overleaf

	background sound (dB)	NA	33.16325	0	-2.72523
		-0.01044	35.18341	2.020163	-2.19329
	Minimum sound (dB)	NA	33.16325	0	-2.72523
		0.019201	35.05135	1.888099	-3.75814
	Maximum sound (dB)	NA	33.16325	0	-2.72523
		-0.00609	35.10236	1.939112	-2.21498
MOVE	Distance to fence	NA	10.77174	0	-4.49754
		-0.00156	12.82002	2.048289	-4.46586
	No. daily visitors	NA	10.77174	0	-4.49754
		-2.46E-05	12.81923	2.047491	-4.47037
	No. People in 10m radius	NA	10.77174	0	-4.49754
		0.005522	12.78621	2.01447	-4.61069
	No. Photographers in 10m	NA	10.77174	0	-4.49754
		0.083298	12.7251	1.953365	-4.66817
	No. children in 10m	NA	10.77174	0	-4.49754
		0.053487	12.63404	1.862305	-4.62097
	background sound (dB)	NA	10.77174	0	-4.49754
		-0.01156	12.81437	2.042638	-3.90869
	Minimum sound (dB)	NA	10.77174	0	-4.49754
		0.015409	12.80152	2.029784	-5.32605
	Maximum sound (dB)	NA	10.77174	0	-4.49754
		-0.0125	12.73694	1.9652	-3.45956
MOTHER-PUP BEHAVIOUR	Distance to fence	NA	34.44881	0	-3.20103
		-0.0055	36.45265	2.003842	-3.09121
	No. daily visitors	NA	34.44881	0	-3.20103
		1.82E-05	36.49448	2.045666	-3.22153
	No. People in 10m radius	NA	34.44881	0	-3.20103
		0.004036	36.43624	1.987433	-3.28236
	No. Photographers in 10m	NA	34.44881	0	-3.20103
		0.023843	36.47466	2.025848	-3.24652
	No. children in 10m	NA	34.44881	0	-3.20103
		-0.0427	36.35843	1.909618	-3.12948
	background sound (dB)	NA	34.44881	0	-3.20103
		0.03237	36.30696	1.858146	-4.862
	Minimum sound (dB)	NA	34.44881	0	-3.20103
		0.057979	35.53859	1.089778	-6.35243
	Maximum sound (dB)	NA	34.44881	0	-3.20103
		0.005811	36.42956	1.980751	-3.69438
NURSING	Distance to fence	NA	40.08121	0	-3.30501
		0.024712	41.13269	1.051487	-3.85962
	No. daily visitors	NA	40.08121	0	-3.30499
		1.53E-05	42.1282	2.046997	-3.32222
	No. People in 10m	NA	40.08121	0	-3.30499

*Continued Overleaf

	radius				
		-0.00195	42.11902	2.03782	-3.26779
	No. Photographers in 10m	NA	40.08121	0	-3.30499
		-0.08924	41.90045	1.819248	-3.15321
	No. children in 10m	NA	40.08121	0	-3.30499
		-0.0726	41.82828	1.747074	-3.19106
	background sound (dB)	NA	40.08121	0	-3.30499
		-0.04002	41.88667	1.805469	-1.27667
	Minimum sound (dB)	NA	40.08121	0	-3.30499
		-0.03115	41.88963	1.808424	-1.65064
	Maximum sound (dB)	NA	40.08121	0	-3.30499
		-0.00543	42.07859	1.997381	-2.84967
PUP-CHECK	Distance to fence	-0.04773	17.49096	0	-2.47427
		NA	17.78415	0.293194	-3.29527
	No. daily visitors	NA	17.78415	0	-3.29527
		9.34E-05	19.73741	1.953255	-3.40459
	No. People in 10m radius	NA	17.78415	0	-3.29527
		-0.00583	19.73738	1.953231	-3.18757
	No. Photographers in 10m	NA	17.78415	0	-3.29527
		-0.09196	19.58843	1.804278	-3.13922
	No. children in 10m	NA	17.78415	0	-3.29527
		-0.01519	19.81329	2.029142	-3.26801
	background sound (dB)	NA	17.78415	0	-3.29527
		-0.03991	19.58875	1.8046	-1.27264
	Minimum sound (dB)	NA	17.78415	0	-3.29527
		0.038887	19.43672	1.652568	-5.39859
	Maximum sound (dB)	NA	17.78415	0	-3.29527
		-0.00984	19.66527	1.881118	-2.47458
REST	Distance to fence	0.017207	107.8485	0	0.209863
		NA	108.509	0.660515	0.553516
	No. daily visitors	NA	108.509	0	0.553516
	No. People in 10m radius	NA	108.509	0	0.553516
		-0.00113	110.5307	2.021705	0.575523
	No. Photographers in 10m	0.007879	108.509	0	0.553516
		NA	110.5434	2.034442	0.538889
	No. children in 10m	NA	108.509	0	0.553516
		-0.00697	110.5246	2.015592	0.56663
	background sound (dB)	NA	108.509	0	0.553516
		0.015507	110.2982	1.789255	-0.23714
	Minimum sound (dB)	NA	108.509	0	0.553516
		-0.02406	109.5509	1.041898	1.84377
	Maximum sound (dB)	NA	108.509	0	0.553516
		0.008569	109.6712	1.162205	-0.16686
Threat	Distance to fence	NA	16.45849	0	-4.03355

*Continued Overleaf

		0.013199	18.37318	1.914696	-4.3173
	No. daily visitors	NA	16.45849	0	-4.03354
		5.82E-05	18.48989	2.031401	-4.1005
	No. People in 10m radius	NA	16.45849	0	-4.03354
		0.003876	18.48186	2.02337	-4.11165
	No. Photographers in 10m	NA	16.45849	0	-4.03354
		0.087567	18.3402	1.881713	-4.21357
	No. children in 10m	NA	16.45849	0	-4.03354
		-0.01138	18.50206	2.043568	-4.01293
	background sound (dB)	NA	16.45849	0	-4.03354
		0.037647	18.3894	1.930909	-5.96769
	Minimum sound (dB)	NA	16.45849	0	-4.03354
		0.008794	18.49789	2.039398	-4.50549
	Maximum sound (dB)	NA	16.45849	0	-4.03354
		-0.00049	18.50765	2.049162	-3.99203
VIGILANCE	Distance to fence	-0.02066	62.38629	0	-0.98368
		NA	62.89427	0.507974	-1.3838
	No. daily visitors	-1.34E-05	62.89427	0	-1.38381
		NA	64.93523	2.040963	-1.36885
	No. People in 10m radius	NA	62.89427	0	-1.38381
		8.72E-06	64.94365	2.04938	-1.38397
	No. Photographers in 10m	NA	62.89427	0	-1.38381
		0.004848	64.93969	2.04542	-1.39284
	No. children in 10m	NA	62.89427	0	-1.38381
		0.02164	64.69189	1.797623	-1.42596
	background sound (dB)	NA	62.89427	0	-1.38381
		-0.02143	64.60419	1.709922	-0.29291
	Minimum sound (dB)	NA	62.89427	0	-1.38381
		0.018737	64.51896	1.624697	-2.3901
	Maximum sound (dB)	NA	62.89427	0	-1.38381
		-0.00972	64.16849	1.27422	-0.57031
MORQ	Distance to fence	NA	1701.946	0	2.861468
	No. daily visitors	NA	1701.946	0	2.861468
	No. People in 10m radius	NA	1701.946	0	0.960667
	No. Photographers in 10m	NA	1701.946	0	2.861468
		-0.19003	1704.432	2.486242	3.214837
	No. children in 10m	NA	1701.946	0	2.861468
		-0.15821	1704.813	2.8672	3.157624
	background sound (dB)	NA	1701.946	0	2.861468
		-0.07073	1706.195	4.248829	6.470453
	Minimum sound (dB)	NA	1701.946	0	2.861468
	Maximum sound (dB)	NA	1701.946	0	2.861468
		-0.05757	1705.843	3.897151	7.715514

3.1.4 FACTORS WHICH MAY INFLUENCE A FEMALES RESPONSE TO A DISTURBANCE

As discussed in section 1.9 there are a number of factors which may influence how females respond to disturbance events. Two factors were selected for analysis in the current study: pup gender and the female's location on the colony.

3.1.4a Pup Gender

The following analyses address question 6, set out in section 1.10.1: Does the pup sex affect female breeding behaviour after a disturbance event? To avoid misrepresentation, only females with pups whose gender could be identified with confidence were included in this comparison. Consequently, sample sizes for data extracted from focal videos in this section are smaller than those in other analyses (indicated in **Table 3.7a**), with 98 focals with female pups (18 females) and 76 focals with male pups (14 females).

With this in mind, the results from this section of the analysis must be considered with caution, as they represent only a small proportion of the whole population. **Table 3.7a** shows a comparison of the main maternal behaviours using generalised linear mixed models (GLMMs). In answer to question 6 outlined in section 1.10.1., the results suggest that pup gender at Donna Nook had a significant impact on the level of alertness, pup checking and vigilance behaviours exhibited by females irrespective of whether or not there were disturbances in the focal. Mothers of male pups spent, on average, more time performing alert, pup checking and vigilance behaviours than mothers of female pups (**Table 3.7a**; **Figure 3.7**). In addition to this, the percentage of the focal (POF) females spent engaged in resting behaviour was affected by the sex of the pup; GLMMs revealed that mothers of male pups spent significantly less time resting than female pups and spent significantly more time in threatening behaviours (**Table 3.7a**). For all other behavioural categories, pup sex did not significantly impact the POF females spent engaged in that behaviour.

In order to identify whether pup sex affected a females response to a disturbance event separate GLMMs were constructed for each behaviour. When disturbance contexts are taken into consideration, in the presence of aircraft disturbance, mothers of male pups perform more pup checking, alert and vigilance behaviours than those of female pups (**Table 3.7b**).None of the other behaviour categories showed any significant differences in response to aircraft disturbance between mothers of male and female pups. When

pedestrian disturbances were present in a focal, mothers of male pups, once again showed significantly higher levels of pup-checking, alert and vigilance responses than mothers of female pups. Pup sex was not a determining factor in the POF females spent engaged in any of the other behavioural categories (**Table 3.7b**). In the case of intraspecific aggressive encounters, females of male pups once again had significantly higher levels of pup-checking, alert and vigilance responses than mothers of female pups. In addition to this, mothers of male pups had higher MORQ scores than mothers of female pups in the presence of pedestrian disturbances, indicating that mothers of male pups showed more affiliative behaviours towards their pups in the presence of pedestrian disturbances than mothers of female pups. Furthermore, females with male pups, spent significantly less time resting than mothers of female offspring in focals where intraspecific aggression was present (**Table 3.7b**). Finally in the presence of natural disturbances, mother of male pups spent significantly more time performing alert behaviours and significantly less time resting when compared with mothers with female offspring. Once again, the results of GLMMs indicate that pup sex is not a determining factor which predicts the POF females spent engaged in any of the other behavioural categories in the presence of natural disturbance (**Table 3.7b**).

Table 3.7a. Outcomes of GLMMs comparing the behaviour of mothers of male pup, with that of mothers of female pups. Disturbance contexts are not taken into account in this analysis. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ΔAICc score give the difference between a particular model and the best model (with the best model scoring a 0). Higher ΔAICc scores indicate models which do a worse job of fitting the data. A ΔAICc score limit of 6 was placed in accordance with Richard, 2008. Factors in the GLMM were pup sex, and ID (as a random factor). "NA" refers to factors which did not significantly affect the percentage of the focal (POF) individuals spent in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. pup-check, alert, comfort etc.

EFFECT OF PUP SEX	Behaviour	Pup Sex Effect	AICc	ΔAICc	Intercept
	PUP-CHECK	+	10.89153	0	-3.5058
		NA	12.32631	1.434781	-4.60899
	ALERT	+	35.35277	0	-1.79885
		NA	35.99796	0.645191	-2.56959
	MOVEMENT	NA	8.879217	0	-4.48092
		+	10.94965	2.070436	-4.50627
	COMFORT	NA	20.31069	0	-2.88307
		+	22.38057	2.069886	-2.85722
	REST	+	70.33157	0	0.692842
		NA	70.39117	0.059601	1.359826
	THREAT	NA	12.32937	0	-4.1207
		+	14.3181	1.988734	-4.6323
	NURSING	NA	28.9744	0	-3.24305
	MOTEHR-PUP BEHAVIOUR	NA	27.26019	0	-3.07322

*Continued Overleaf

	VIGILANCE	+	39.19486	0	-2.45497
		NA	39.26445	0.069593	-1.57756
	MORQ	NA	1114.457	0	2.329318

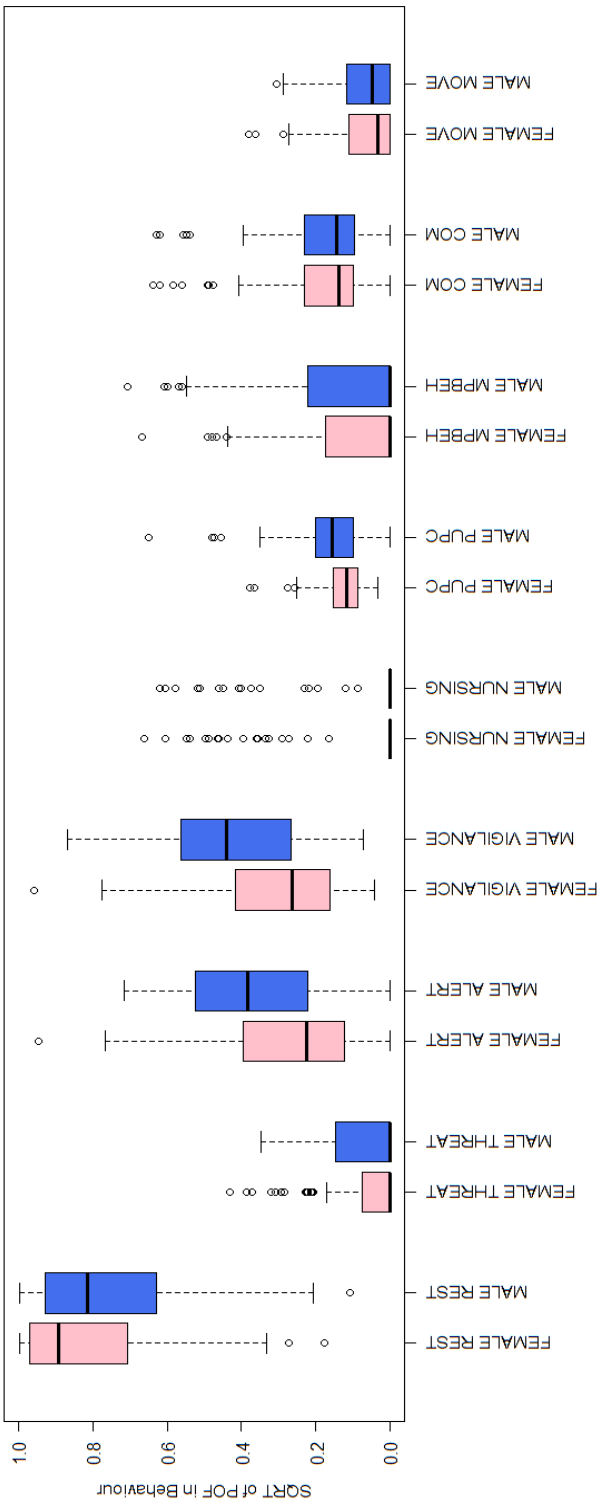

Figure 3.7.: The effect of pup sex on behaviour. Percentage of the focal (POF) (shown as the SQRT of POF) that an individual spent performing each of the broad category behaviours analysed in this study. The symbol represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The square-root (SQRT) of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data. The vigilance behaviour is a summation of the time spent in alert and pup-check behaviours in the focal.
The focals are separated into those with male and female pups.

Table 3.7b. Outcomes of GLMMs comparing the behaviour of mothers of male and female pups, with disturbance contexts taken into consideration. Comparison of the sexes was made in videos where the disturbances were present. The left hand column describes the disturbance being investigated. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ΔAICc score give the difference between a particular model and the best model (with the best model scoring a 0). Higher ΔAICc scores indicate models which do a worse job of fitting the data. A ΔAICc score limit of 6 was placed in accordance with Richard, 2008. Factors included in the GLMM were pup sex, and ID (as a random factor). "NA" refers to factors which did not significantly affect the percentage of the focal (POF) individuals spent in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. pup-check, alert, comfort etc. and for each mode of disturbance e.g. aircraft, people etc.

Male-Female pup comparison in disturbed conditions	Behaviour	Pup Sex Effect	AICc	ΔAICc	INT
Aircraft	PUP-CHECK	+	5.839919	0	-3.72835
		NA	7.903534	2.063615	-4.29858
	ALERT	+	19.69776	0	-1.88709
		NA	21.10584	1.40808	-2.66723
	MOVEMENT	NA	6.76109	0	-4.4317
		+	8.856779	2.095689	-5.0511
	COMFORT	NA	10.97995	0	-2.86461
		+	13.11322	2.133265	-2.99564
	REST	NA	30.27578	0	0.801177
		+	31.94138	1.665601	1.254683
	THREAT	NA	9.259942	0	-3.818
		+	11.38574	2.125797	-4.09124
	NURSING	NA	15.64134	0	-3.4646
		+	17.71075	2.06941	-2.98596
	MOTEHR-PUP BEHAVIOUR	NA	14.41897	0	-3.26947
		+	16.51952	2.100548	-2.93857
	VIGILANCE	+	19.42896	0	-1.69556
		NA	20.72754	1.298574	-2.47559
	MORQ	NA	555.5988	0	2.763647
		+	555.8602	0.261422	2.855388
People	PUP-CHECK	+	10.32496	0	-3.46415
		NA	11.61102	1.286056	-4.8041
	ALERT	+	30.06217	0	-2.83844
		NA	30.06574	0.003568	-1.80908
	MOVEMENT	NA	7.822784	0	-4.53045
		+	9.864929	2.042145	-5.00683
	COMFORT	NA	18.27843	0	-2.82609
		+	20.36118	2.082749	-2.82732
	REST	+	59.97845	0	1.481227
		NA	60.57076	0.59231	0.650215
	THREAT	NA	11.44012	0	-4.07039
		+	13.46571	2.025587	-4.52148
	NURSING	NA	26.88768	0	-3.15371
		+	28.96827	2.080595	-3.09815
	MOTEHR-PUP	NA	24.31505	0	-3.00542

*Continued Overleaf

	BEHAVIOUR				
		+	26.27322	1.95816	-3.40741
	VIGILANCE	+	32.75886	0	-2.72627
		NA	33.70549	0.946632	-1.57786
	MORQ	NA	899.2326	0	2.607618
		+	899.6203	0.387779	2.533752
Aggression	PUP-CHECK	+	8.816506	0	-3.29307
		NA	10.423	1.606492	-4.54621
	ALERT	+	22.44913	0	-1.51181
		NA	23.85198	1.402843	-2.18583
	MOVEMENT	NA	6.948623	0	-4.3274
		+	9.07423	2.125607	-4.4458
	COMFORT	NA	14.6871	0	-2.80791
		+	16.76213	2.07503	-2.51695
	REST	+	42.34031	0	0.442514
		NA	42.77827	0.437961	1.243412
	THREAT	NA	9.401118	0	-3.74765
		+	11.52016	2.119039	-3.93269
	NURSING	NA	16.30299	0	-3.41335
		+	18.40738	2.104391	-3.67417
	MOTEHR-PUP BEHAVIOUR	NA	18.28366	0	-2.95856
		+	19.73784	1.45418	-4.17397
	VIGILANCE	+	24.91857	0	-1.2863
		NA	25.79212	0.873547	-2.11792
	MORQ	+	673.4609	0	3.255519
		NA	674.1207	0.659757	3.608544
Natural	PUP-CHECK	NA	5.183186	0	-3.76009
		+	7.296801	2.113615	-4.49741
	ALERT	+	14.61834	0	-1.7794
		NA	15.73544	1.1171	-2.89876
	MOVEMENT	NA	6.069821	0	-4.30841
		+	8.262724	2.192903	-4.04659
	COMFORT	NA	7.194001	0	-3.32772
		+	9.187327	1.993326	-4.26997
	REST	NA	23.36879	0	0.736974
		+	24.6882	1.319413	1.470749
	THREAT	NA	6.256919	0	-4.37679
		+	8.405033	2.148114	-5.14861
	NURSING	NA	15.04066	0	-2.99096
		+	17.06672	2.026063	-2.2925
	MOTEHR-PUP BEHAVIOUR	NA	12.89467	0	-2.99521
		+	15.09052	2.19585	-3.1033
	VIGILANCE	+	14.74076	0	-1.60611
		NA	15.73529	0.994526	-2.7147
	MORQ	NA	427.1109	0	4.059234
		+	428.5775	1.466613	3.340351

3.1.4b Female Nursing Location

The following analyses address question 4, set out in section 1.10.1: Is there an observable difference in the behaviour of those females which choose to give birth near to the RAF site and those which give birth near to the public car park? And does the choice of birthing site effect a female's response to a disturbance?

There are a number of significant differences in the behavioural repertoires of those females which give birth close to RAF base and those which give birth close to the public car park (**Figure 3.8**). In answer to the question set out in 1.10.1. as to whether there is a difference in behaviour between females which give birth at different locations within the colony the generalised linear mixed model (GLMMs) analysis indicates that females at the RAF site spent significantly more time engaging in alert and vigilance behaviours than those at the car park site (**Table 3.8a**). In addition to this, females which gave birth close to the RAF site spent significantly more time engaging in threatening behaviours than females which gave birth near to the public car park. Furthermore, GLMMs revealed that the mother-offspring relationship quality (MORQ) scores of females which gave birth closer to the RAF base were higher than those at the car park site, indicating that females which pupped towards the RAF base exhibited higher levels of affiliative behaviour towards their pup than females which gave birth close to the Stonebridge car park. For all of the other behavioural categories investigated in this study, pupping location was not found to be a significant influence on behaviour (**Figure 3.8**).

The car park and RAF sites had similar levels of aircraft, pedestrian and intraspecific aggressive disturbances (**Table 3.8b**), however the results of Mann-Whitney U analysis which compared disturbance rates at the two sites suggest that the site close to the RAF base was subjected to significantly higher levels of natural disturbances than in the focals which took place nest to the Stonebridge car park ($U = 7751$, $p = 0.00021$).

Using separate GLMMS to discern whether when disturbance contexts are taken into account reveals some further interesting differences between the behaviours of those females located close to the RAF site and those located near to the public car park. In those focals in which aircraft disturbances were present, females close to the RAF base exhibited significantly higher levels of alert and vigilance behaviour than those female located at the car park site (**Table 3.8c**). The location of the female was not a significant factor which affected the POF females spent engaged in any of the other behavioural categories when aircraft disturbances were present. In those focals where

pedestrian disturbances were present, females at the RAF site spent significantly more time engaged in alert behaviours than those females which gave birth close to the public car park. Other than alert behaviours, none of the other behavioural categories varied between the RAF and car park site when pedestrian disturbances were present. With regard to focals in which natural disturbances were present; females who pupped close to the RAF base spent significantly longer engaged in alert and comfort movement behaviours than those females who pupped close to the car park (**Table 3.8c**). Once again the duration of time females spent in all other behavioural categories investigated in this study did not vary between the two sites when natural disturbances were present. Finally, the location of females was not a determining factor in the POF females spent engaged in any of the behavioural categories in focals in which intraspecific aggressive encounters occurred.

Table 3.8a. Outcomes of generalised linear mixed models (GLMMs) comparing the behaviour of mothers who gave birth at close to the RAF base and those which gave birth close to the public car park (CP), irrespective of the disturbance context. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ΔAICc score give the difference between a particular model and the best model (with the best model scoring a 0). Higher ΔAICc scores indicate models which do a worse job of fitting the data. A ΔAICc score limit of 6 was placed in accordance with Richard, 2008. Factors included in the GLMM were location, and ID (as a random factor). "NA" refers to factors which did not significantly affect the percentage of the focal (POF) individuals spent in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. pup-check, alert, comfort etc.

Effect of Location	Behaviour	Location Effect	AICc	ΔAICc	INT
	PUP-CHECK	NA	17.78415	0	-3.29527
		+	19.83315	2.048998	-3.29938
	ALERT	+	53.08713	0	-1.62393
		NA	53.4545	0.36737	-1.49681
	MOVEMENT	NA	10.77174	0	-4.49754
		+	12.72503	1.953296	-4.39132
	COMFORT	NA	33.16325	0	-2.72525
		+	35.15331	1.990058	-2.76588
	REST	NA	108.509	0	0.553516
		+	110.3514	1.842376	0.51692
	THREAT	+	16.45849	0	-4.03355
		NA	18.31525	1.856767	-3.91473
	NURSING	NA	40.08121	0	-3.30501
		+	41.20588	1.124679	-3.54093
	MOTEHR-PUP BEHAVIOUR	NA	34.44881	0	-3.20103
		+	36.29527	1.846459	-3.29782
	VIGILANCE	+	62.89427	0	-1.3838
		NA	63.54354	0.649278	-1.27447
	MORQ	+	1701.467	0	3.085511
		NA	1701.946	0.478669	2.861468

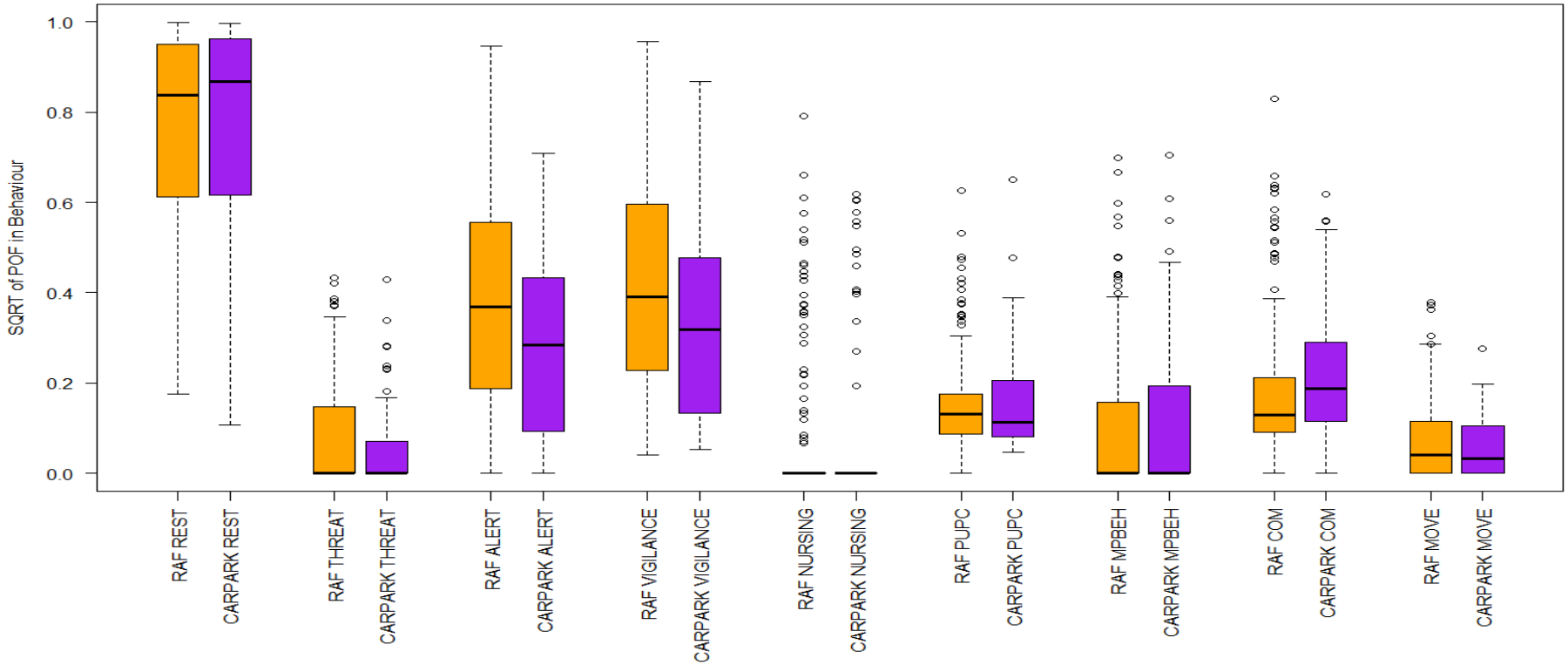

Figure 3.8.: The effect of birthing site on behaviour. POF of random focal videos (shown as the SQRT of POF) spent performing the broad scale behaviours analysed in this study. The focals are separated into those with male and female pups. The symbol ○ represents an outlier. Purple bars represent those females which chose to give birth near to the public car park and oange bars represent those females which pupped near to the RAF base. Both sites were accessible by the genereal public. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The square-root (SQRT) of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data. The vigilance behaviour is a summation of the time spent in alert and pup-check behaviours in the focal.

Table 3.8b. Outcomes of Mann-Whitney U test comparing the disturbance rates between the two study sites, the RAF base and those which chose to give birth close to the public car park (CP).

Comparing disturbance rates between car park and RAF base	Disturbance	Median	Mann u value	P VALUE
N. Females= 49	PEOPLE	0.005714	5380	0.1345
N. Focals=249	AGG	0.006096	6324.5	0.6741
	NAT	**0**	**7751**	**0.00021**
	AIRCRAFT	0	6911	0.093

Table 3.8c. Outcomes of the generalised linear mixed models (GLMM) tests, which compared the behaviour of mothers who gave birth at close to the RAF base and those who gave birth close to the public car park (CP). Disturbance contexts were taken into consideration. Comparisons were made in videos where the disturbances were present. The left hand column describes the disturbance being investigated. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ∆AICc score give the difference between a particular model and the best model (with the best model scoring a 0). Higher ∆AICc scores indicate models which do a worse job of fitting the data. A ∆AICc score limit of 6 was placed in accordance with Richard, 2008. Factors included in the GLMM were location, and ID (as a random factor). "NA" refers to factors which did not significantly affect the percentage of the focal (POF) individuals spent in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. pup-check, alert, comfort etc. and for each mode of disturbance e.g. aircraft, people etc.

Disturbance category	Behaviour	Location	AICc	∆AICc	INT
Aircraft	PUP-CHECK	NA	7.862561	0	-3.59153
		+	9.925692	2.063131	-3.65696
	ALERT	+	28.52187	0	-1.7104
		NA	29.4331	0.911227	-1.56906
	MOVEMENT	NA	7.782164	0	-4.45333
		+	9.826919	2.044754	-4.3492
	COMFORT	NA	15.00947	0	-2.81646
		+	16.4445	1.435032	-3.02444
	REST	NA	46.35475	0	0.702698
		+	48.26067	1.905927	0.655243
	THREAT	NA	11.40099	0	-3.83975
		+	13.23996	1.838971	-3.68257
	NURSING	NA	21.11706	0	-3.48504
		+	23.07704	1.959974	-3.60932
	MOTEHR-PUP	NA	16.92834	0	-3.40523
		+	18.9958	2.067463	-3.35373
	VIGILANCE	+	30.44592	0	-1.51683
		NA	31.69661	1.250694	-1.40192
	MORQ	NA	769.148	0	2.544298
		+	769.3169	0.168904	2.584121
People	PUP-CHECK	NA	17.78415	0	-3.29527
		+	19.83315	2.048998	-3.29938
	ALERT	+	53.08713	0	-1.62393
		NA	53.4545	0.36737	-1.49681
	MOVEMENT	NA	10.77174	0	-4.49754

*Continued Overleaf

		+	12.72503	1.953296	-4.39132
	COMFORT	NA	33.16325	0	-2.72525
		+	35.15331	1.990058	-2.76588
	REST	NA	108.509	0	0.553502
		+	110.3514	1.842376	0.516915
	THREAT	NA	16.45849	0	-4.03354
		+	18.31525	1.856767	-3.91473
	NURSING	NA	40.08121	0	-3.30499
		+	41.20588	1.124679	-3.54093
	MOTEHR-PUP	NA	34.44881	0	-3.20106
		+	36.29527	1.846459	-3.29782
	VIGILANCE	NA	62.89427	0	-1.38381
		+	63.54354	0.649278	-1.27446
	MORQ	NA	1701.467	0	3.085511
		+	1701.946	0.478669	2.861468
Aggression	PUP-CHECK	NA	17.78415	0	-3.29527
		0.014963	19.83315	2.048998	-3.31435
	ALERT	NA	53.08713	0	-1.62393
		-0.53019	53.4545	0.36737	-0.96663
	MOVEMENT	NA	10.77174	0	-4.49754
		-0.45611	12.72503	1.953296	-3.93523
	COMFORT	NA	33.16325	0	-2.72525
		0.141742	35.15331	1.990058	-2.90763
	REST	NA	108.509	0	0.553502
		0.135311	110.3514	1.842376	0.38159
	THREAT	NA	16.45849	0	-4.03354
		-0.5211	18.31525	1.856767	-3.39366
	NURSING	NA	40.08121	0	-3.30499
		0.688182	41.20588	1.124679	-4.22913
	MOTEHR-PUP	NA	34.44881	0	-3.20106
		0.317868	36.29527	1.846459	-3.61571
	VIGILANCE	NA	62.89427	0	-1.38381
		-0.4406	63.54354	0.649278	-0.83386
	MORQ	NA	1701.467	0	3.085511
		+	1701.946	0.478669	2.861468
Natural	PUP-CHECK	NA	6.2256	0	-3.66171
		+	8.30709	2.08149	-3.60287
	ALERT	+	20.32385	0	-1.64179
		NA	21.77166	1.447809	-1.55313
	MOVEMENT	NA	6.598793	0	-4.37209
		+	8.569187	1.970394	-4.24546
	COMFORT	+	11.85063	0	-3.15842
		NA	13.81893	1.968303	-3.25701
	REST	NA	34.38801	0	0.638369
		+	35.83438	1.446373	0.565548
	THREAT	NA	7.326194	0	-4.18215
		+	9.30215	1.975956	-4.06557
	NURSING	NA	19.30073	0	-3.05961
		+	21.41524	2.11451	-3.09032
	MOTEHR-PUP	NA	16.55205	0	-3.06053
		+	18.42489	1.872835	-2.967
	VIGILANCE	NA	21.16548	0	-1.46787
		+	22.53908	1.373604	-1.37908
	MORQ	NA	597.3465	0	2.871701
		+	598.0556	0.709176	2.871057

3.1.5 CIDs in a female's response to disturbance

The following analyses address question 7, set out in section 1.10.1: Are there consistent individual differences (CIDs) in behaviour between mothers, when compared between disturbed and non-disturbed conditions? Non-disturbed conditions did not contain aircraft disturbances and had the least amount of natural, pedestrian and intra-specific disturbances as possible. Low levels of the three other disturbance categories (pedestrian, intra-specific aggression and natural disturbances) were not included as "disturbances" in this analysis due to the fact that they were present in small amounts in the majority of focals. In addition to this, aircraft disturbances caused the greatest change in ambient noise level (measured in decibels) when compared with the three other behavioural categories. The intra-class correlation coefficient (ICC) was used to assess the existence (if any) of CIDs amongst maternal behaviours across late and early lactation and disturbed and non-disturbed conditions. Categories of maternal behaviours assessed are listed with their ICC value, test statistic and p value in **Table 3.9**. In this section, sample size (N) of a category (e.g. mothers with male pups and female pups, mothers at RAF and car park sites) refers to the number of focal videos used for the study. This sample size number is not equal to the number of females included in that category. This is due to the fact that multiple video focals for each female were taken throughout the study.

When ICCs were compared across early and late lactation but were context specific (e.g. separated into disturbed and non-disturbed focals) in terms of disturbance; in non-disturbed conditions, the results of the ICCs suggest the presence of CIDs with respect to alert, pup checking and vigilance behaviours (Alert ICC=0.339, $F_{26,27}$ =2.03, p =0.037*; PUPC:* ICC =0.525, $F_{26,27}$ =3.21, p =0.0018; Vigilance ICC =0.485, $F_{26,27}$ =2.89, p =0.0039; **Figure 3.9.a,b**). In comparison, in disturbed conditions, the results of the ICC reveal that only pup-checking and comfort behaviours are consistent across lactation stages (*PUPC:* ICC =0.719, $F_{26,27}$ =6.12, p =<0.001; Comfort ICC =0.452, $F_{26,27}$ =2.65, p =0.0071). All other behaviour categories showed no indication of consistent individual differences (CIDs) across lactation stages within disturbance contexts. Categories of maternal behaviours examined in this analysis are listed with their ICC values, test statistic and p value in **Table 3.9**.

After the separate ICCs were analysed for each disturbance context, an ICC which incorporated both early and late lactation stages as well as disturbed and non-disturbed conditions was constructed in order to see whether CID in behaviour were

noted across disturbance contexts. The ICCs which were constructed across disturbance contexts indicated the presence of CIDs in the time individuals spent on Alert behaviours (ICC =0.274, $F_{26, 81}$ =2.51, p =<0.001) and also the time individuals spent engaged in pup checking behaviours (ICC =0.683, $F_{26, 81}$ =9.63, p =<0.001; **Table 3.10**). The significance of the CIDs increases when alert and pup checking behaviours are considered together as vigilance behaviours (ICC = 0.422, $F_{26, 81}$ =3.92, p = <0.001; **Figure 3.10**). ICCs also suggest the presence of CIDs with respect to rest and comfort behaviours, with both the POF of rest and comfort behaviours showing consistency both across lactation stages and across disturbance conditions (rest ICC =0.28, $F_{26,81}$ =2.56, p =<0.001*;* comfort*:* ICC =0.182, $F_{26,81}$ =1.89, p =0.0162). However nursing, MPBEH, active movement and threat behaviours were not consistent across disturbance behavioural categories suggest that these behaviours are context specific.

Table 3.9: ICC statistics for identification of consistent individual differences (CIDs) in maternal behaviour across lactation stages but separated into the two disturbance contexts. "N" denotes the sample size of females. Degrees of freedom represented by subscript beside F value. Those values highlighted in bold represent significant results at the 0.05 p-value level. A negative ICC value indicates a negative correlation. Each row represents one ICC.

	Behaviour	**ICC**	**F Value (df)**	**P-value**	**Confidence intervals**	
					lower	upper
Non-Disturbed	N=28					
	MORQ	0.0218	1.04(26,27)	0.455	-0.35	0.39
	ALERT	**0.339**	**2.03(26,27)**	**0.037**	**-0.035**	**0.631**
	PUPC	**0.525**	**3.21(26,27)**	**0.0018**	**0.193**	**0.75**
	REST	0.101	1.23(26,27)	0.301	-0.278	0.456
	VIGILANCE	**0.485**	**2.89(26,27)**	**0.0039**	**0.141**	**0.726**
	Threat	-0.123	0.782(26,27)	0.734	-0.471	0.261
	MOTEHR-PUP	0.1	1.22(26,27)	0.304	-0.28	0.455
	Nursing	-0.072	0.865(26,27)	0.643	-0.43	0.308
	Comfort	0.308	1.89(26,27)	0.0529	-0.069	0.61
	Movement	-0.17	0.709(26,27)	0.808	-0.508	0.215
Disturbed	N=28					
	MORQ	0.155	1.37(26,27	0.212	-0.227	0.498
	ALERT	0.119	1.27(26,27)	0.27	-0.262	0.47
	PUPC	**0.719**	**6.12(26,27)**	**>0.001**	**0.476**	**0.861**
	REST	0.291	1.82(26,27)	0.0641	-0.088	0.598
	VIGILANCE	0.288	1.81(26,27)	0.066	-0.091	0.596
	Threat	-0.0556	0.895(26,27)	0.611	-0.416	0.323
	MOTEHR-PUP	-0.0446	0.915(26,27)	0.589	-0.407	0.333
	Nursing	-0.1	0.818(26,27)	0.695	-0.453	0.282
	Comfort	**0.452**	**2.65(26,27)**	**0.0071**	**0.1**	**0.706**
	Movement	-0.178	0.697(26,27)	0.819	-0.514	0.207

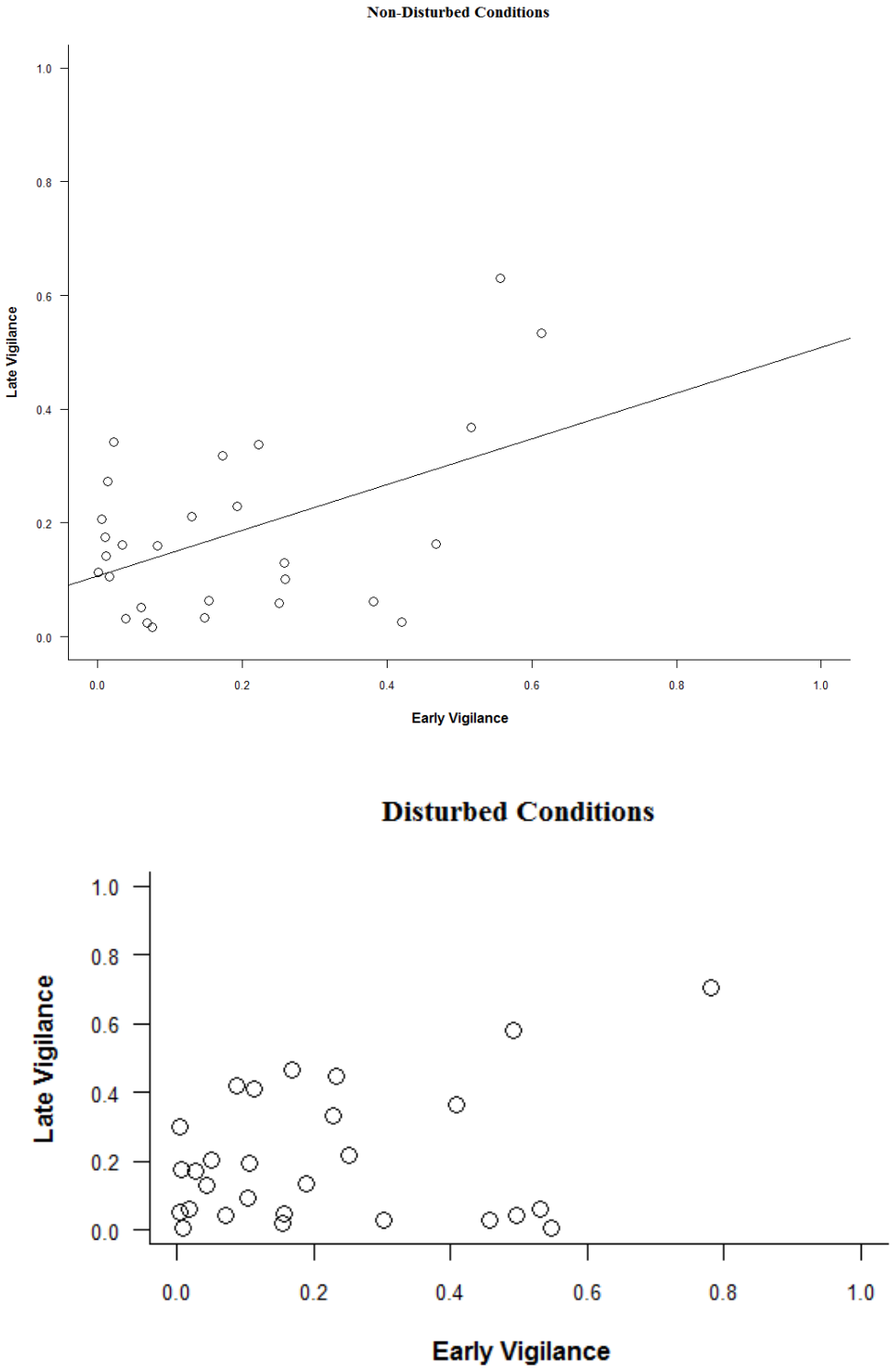

Figure 3.9 *a.* POF in focal videos spent on vigilance behaviours in focals with no disturbance; *b.* POF in focal videos spent on vigilance behaviours in focals with disturbance. Each point represents an individually known female.

Table 3.10: ICC statistics for identification of CIDs in maternal behaviour across: early non-disturbed conditions; early disturbed conditions; late non-disturbed conditions; and late disturbed conditions. "N" denotes the sample size of females. Degrees of freedom represented by subscript beside F value. Those values highlighted in bold represent significant results at the 0.05 p-value level. A negative ICC value indicates a negative correlation. Each row represents one ICC.

	Behaviour	ICC	F Value (df)	P-value	Confidence intervals	
					lower	upper
N=28						
	MORQ	0.105	$1.47_{(26,81)}$	0.0984	-0.047	0.324
	ALERT	**0.274**	**$2.51_{(26,81)}$**	**> 0.001**	**0.091**	**0.499**
	PUP-CHECK	**0.683**	**$9.63_{(26,81)}$**	**>0.001**	**0.522**	**0.819**
	REST	**0.28**	**$2.56_{(26,81)}$**	**>0.001**	**0.097**	**0.506**
	Vigilance	**0.422**	**$3.92_{(26,81)}$**	**>0.001**	**0.229**	**0.629**
	Threat	-0.0657	$0.754_{(26,81)}$	0.79	-0.169	0.111
	MOTHER-PUP	0.0462	$1.19_{(26,81)}$	0.269	-0.091	0.256
	Nursing	0.0523	$1.22_{(26,81)}$	0.246	-0.087	0.263
	Comfort	**0.182**	**$1.89_{(26,81)}$**	**0.0162**	**0.013**	**0.408**
	Movement	-0.0666	$0.75_{(26,81)}$	0.794	-0.17	0.11

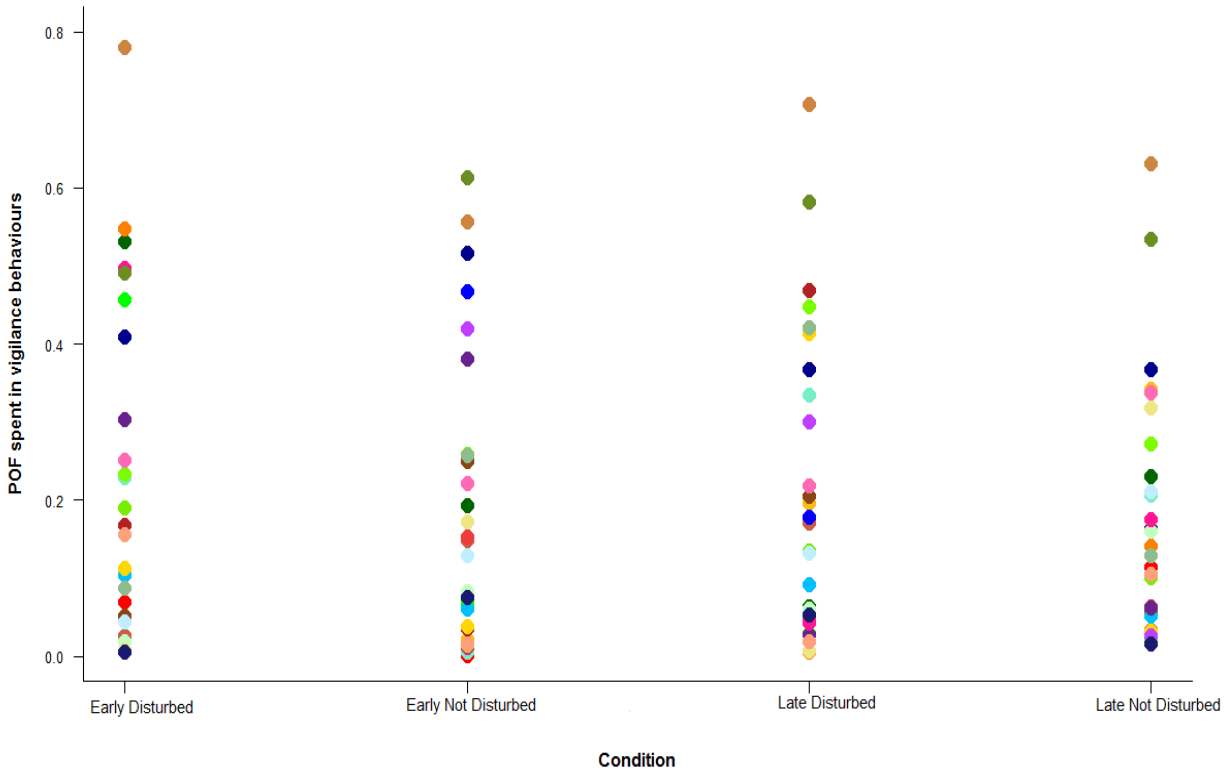

Figure 3.10 *c.* POF in vigilance behaviours in four focal videos across lactation stages and disturbance contexts. Each colour represents an individually known female.

3.2 GLMM ANALYSIS INCORPORATING ALL CONFOUNDING VARIABLES WHICH MAY AFFECT INDIVIDUAL BEHAVIOUR.

3.2.1 Disturbance GLMMS

After the initial exploratory analyses (section 3.1), more specific generalised linear mixed models (GLMM) were built to examine the potential effect of disturbance on behaviour whilst controlling for multiple samples from individuals, and other potentially influential covariates such as weather. The factors included in these models were selected by their significance in earlier simpler models or their significance in prior studies. These larger models ensured that all the variables which were measured on site and were identified as possible factors in influencing maternal behaviour could be included in the analysis; this included disturbance parameters, visitor attendance and auditory parameters, weather parameters, the total number of individuals within five body lengths of the focal female (TI5BL), the pup stage (PS), the day in field (DIF), their location on the colony and MORQ score. These large scale models enabled he study to answer question 2a set out in section 1.10.1. For all GLMMs, all focals were included so the sample size was 249 focals. Although preliminary analysis did indicate that pup sex may be an influencing factor in the behaviour of females, pup sex could not be included as a factor in these GLMMs as females with pups of unknown sex were present in the study. The removal of these females with pups of unknown sex would have caused a dramatic decrease in the sample size of the study and the decision was therefore made to exclude pup sex as a factor in these GLMMs.

The first group of GLMMS investigated the influence of potential disturbances on the percentage of the focal (POF) females spent engaged in certain behaviours. For this analysis a GLMM was run for each of the behaviours in turn. In answer to question 2a in set out in section 1.10.1., the GLMMs revealed that none of the factors investigated in this study significantly affected the POF females spent engaged in active movement, nursing, mother-pup behaviour nor threat behaviours (**Table 3.11**). The GLMM for alert behaviours revealed that the POF females spend in alert behaviours is significantly affected by the day in field and whether the pup was born close to the car park or RAF site. Females which gave birth closer to the RAF base experienced higher POF of alert behaviours per focal than females who chose to give birth closer to the public car park. Females also performed more alert behaviours as the study progressed. GLMMS for pup checking and comfort movements revealed that the only factor which significantly affected these two behaviours was the day in field. Both the duration of pup checking and comfort behaviours significantly increased as the number of days in the field

progressed (**Table 3.11**). With respect to the POF females spent engaged in vigilance behaviours, the factors which were shown to affect this were the location of the females, the day in field and also the stage of the pup. Females spent more time vigilant towards the RAF base, later in the study and with pups of a higher pup stage (**Table 3.11**). Finally, with respect to the POF females spent in resting behaviours, the factors which affect it are once again the location of the females, the day in field and also the stage of the pup. Females with younger pups and those which gave birth close to the Stonebridge car park spent more of their time engaged in resting behaviours than those females with late stage pups and those females which gave birth closer to the RAF base. Females also tended to engage in more resting behaviours earlier in the breeding season (**Table 3.11**).

The second set of GLMMS investigated the factors which may impact upon what the frequency of behaviours in focals. Once again none of the disturbance parameters affected the frequency of any of the behavioural parameters. As with the GLMMs which investigated the POF spent in behaviours, none of the factors investigated in this study significantly affected the frequency of active movement, nursing, threatening and mother-pup behaviours according to the GLMM analysis (**Table 3.12**). With regards to the factors which affected the frequency of alert and vigilance behavioural, the only two significant variables were the location of the female and the day in field. In both cases, females close to the RAF base had higher a higher proportion of scans of vigilance and alert behaviours than those females which gave birth close to the public car park (**Table 3.12**). Females also exhibited a higher frequency of alert and vigilance behaviours later in the breeding season. The only factor which affected the number of comfort and pup checking behaviours was the day in field. There was a positive correlation with day in field and both of these behaviours, with a higher frequency of both seen as the season progressed (**Table 3.12**). Finally, the factors which affected the number of resting behaviours were the location of the female, the day in field, the MORQ score, the mother-pup distance, the pup stage and an interaction factor between pup stage and day in field. Females which gave birth closer to the car park had higher resting rates than those which pupped closer to the RAF base. The frequency of resting behaviours was negatively correlated with day in field and MORQ value, meaning less attentive mother and mothers earlier in the season rested more frequently than more affiliative mothers and mothers who were present as the season progressed. Mothers rested more frequently with pups of a younger pup stage and rested more when within a closer proximity to their pup (**Table 3.12**)

Table 3.11 generalised linear mixed model (GLMM) analysis on the factors which affect the percentage of the focal (POF) females spend in certain behaviours during lactation. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ΔAICc score give the best model (with the best model scoring a 0). Higher ΔAICc scores indicate models which do a worse job of fitting the data. A ΔAICc score limit of 6 was placed. Factors included in the GLMM were POF aircraft, pedestrian, natural and aggressive disturbance, location, temperature, day in field, daily visitor numbers, distance to fence, maximum sound level, ORQ score, mother-pup distance, pup stage, the number of conspecifics in 5 body lengths, visibility and ID (as a random factor). "NA" refers to factors which did not significantly affect the percentage of the focal (POF) individuals spent in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. pup-check, alert, comfort etc.

	AGG	AIR	TEMP	AVGPEOP	CPRAF	DIF	DistFence	MSOUND	MORQ	MPdIST	NAT	PEOP	PS	TI5BL	VIS	DIF:PS	DF	AICc	ΔAICc	INT
ALERT																				
Model 1					+	0.04											4.00	50.71	0.00	-3.05
Model 2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	53.09	2.37	-1.62
Model 3						0.02											3.00	53.20	2.48	-2.05
Model4					+												3.00	53.45	2.74	-2.03
COM																				
Model 1						0.08											3.00	26.52	0.00	-4.44
PUPC																				
Model 1						0.06											3.00	16.36	0.00	-4.63
Model 2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	17.78	1.43	-3.30
REST																				
Model 1					+	-0.08							+				7.00	95.08	0.00	2.17
Model 2						-0.06							+				6.00	98.22	3.14	1.33
Model 3					+	-0.05											4.00	98.71	3.63	2.08
VIG																				
Model 1					+	0.06							+				7.00	56.10	0.00	-3.08
Model 2					+	0.05											4.00	56.70	0.61	-3.03
Model 3						0.05							+				6.00	58.78	2.69	-2.24
Model4						0.03											3.00	60.32	4.23	-2.01
MOVE																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	10.77	0.00	-4.50
NURSE																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	40.08	0.00	-3.31
MPBEH																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	34.45	0.00	-3.20
THREAT																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	16.46	0.00	-4.03

Table 3.12 Outcome of GLMM analysis on the factors which affect the frequency those females exhibited certain behaviours during lactation. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ΔAICc score give the best model (with the best model scoring a 0). Higher ΔAICc scores indicate models which do a worse job of fitting the data. A ΔAICc score limit of 6 was placed. Factors included in the GLMM were POF aircraft, pedestrian, natural and aggressive disturbance, location, temperature, day in field, daily visitor numbers, distance to fence, maximum sound level, ORQ score, mother-pup distance, pup stage, the number of conspecifics in 5 body lengths, visibility and ID (as a random factor). "NA" refers to factors which did not significantly affect the percentage of the focal (POF) individuals spent in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. pup-check, alert, comfort etc.

	AGG	AIR	TEMP	AVGPEOP	CPRAF	DIF	DistFence	MSOUND	MORQ	MPdIST	NAT	PEOP	PS	TI5BL	VIS	DIF:PS	DF	AICc	ΔAICc	INT
ALERT																				
Model 1					+	0.04											4	52.03	0.00	-3.14
Model 2						0.02											3	54.72	2.69	-2.12
Model 3																	2	55.10	3.07	-1.64
Model 4					+												3	55.52	3.49	-2.03
COM																				
Model 1						0.09											3	26.24	0.00	-4.75
MOVE																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2	10.70	0.00	-3.52
THREAT																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2	16.68	0.00	-4.03
REST																				
Model 1					+	-0.15			-0.06	+			+		0.00	+	18.00	302.14	0.00	6.15
Model 2					+	-0.10			-0.05	+			+		0.00		15.00	302.95	0.81	5.50
Model 3					+	-0.14			-0.05	+			+			+	17.00	303.59	1.45	4.75
Model 4					+	-0.11				+			+		0.00		14.00	303.70	1.56	5.29
Model 5					+	-0.15				+			+		0.00	+	17.00	303.72	1.58	5.84
Model 6					+	-0.14				+			+			+	16.00	304.76	2.62	4.55
Model 7						-0.09			-0.05	+			+		0.00		14.00	305.42	3.28	4.82
MPBEH																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2	34.87	0.00	-3.20
NURSE																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2	40.63	0.00	-3.28
PUPC																				
Model 1						0.09											3	15.99	0.00	-5.46
Model 2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2	20.18	4.19	-3.37
VIG																				
Model 1					+	0.054											4	57.45	0.00	-3.25
Model 2						0.038											3	61.87	4.42	-2.17

3.3 INVESTIGATING THE IMMEDIATE RESPONSES OF FEMALE GREY SEALS TO DISTURBANCE EVENTS

The following analyses addresses question 8 set out in section 1.10.1. What are the immediate behavioural effects of disturbance on grey seals? So far the analysis has purely looked at the responses of seals to disturbance events on the scale of the whole focal, however previous studies have revealed that the behavioural responses exhibited by individuals to disturbance events are often short lived (Cassini *et al*., 2004; Wolf and Croft, 2010), and individuals resume their normal behaviour within a few minutes after a disturbance event. This study examined the immediate effects in two ways. First, a generalised linear mixed model (GLMM) for each behavioural category was created in which the behaviours were treated as scan samples and shifted in time. The factors included in this GLMM model were the same as those included in section 3.2.1. Secondly, the duration of behaviours were compared two minutes before and two minutes after each of the four disturbance events using Mann Whitney U analysis.

In answer to question 8 set out in the aims in section 1.10.1, the GLMMs which analysed the immediate effects of disturbances on grey seals revealed that none of the disturbance categories investigated in this study significantly affected the duration that females spent in active movement, nursing, mother-pup behaviour nor threat behaviours (**Table 3.13**). The GLMM run on alert behaviours revealed that alert and vigilance behaviours after disturbance events are affected by the day in field and whether the pup was born close to the car park or RAF site. Once again females close to the RAF base had higher rates of alert and vigilance behaviours after disturbance events than those females closer to the public car park. Females also had significantly higher rates of alert behaviours later on in the field season **(Table 3.13).** The GLMMs for comfort movements and pup checking behaviours revealed that the only factor investigated in this study which affected the rate of comfort movements and the amount of pup-checking behaviour under time lag conditions was the day in field. Once again females had significantly higher rates of comfort movements and pup checking behaviours later in the breeding season (**Table 3.13**).Finally the analysis of resting behaviours under a ten second time lag revealed that the factors which affect resting behaviours was the day in field and female location. Females tended to spend more time engaged in resting behaviours earlier in the breeding season and those females who lay close to the public car park engaged in significantly higher levels of resting behaviour than those females which chose to rest near to the RAF base (**Table 3.13**).

Comparisons of behaviour two minutes before and after aircraft disturbances using Mann Whitney U analysis indicated that individuals may alter their resting behaviour patterns after disturbance events. Mann Whitney U analysis indicated that females on average spent significantly more time engaged in rest behaviours in the two minutes preceding a disturbance event than after an aerial disturbance event (**Figure 3.14a; Table 3.14**). The differences in the duration of all of the other behavioural categories before and after an aircraft disturbance were statistically insignificant (**Figures 3.14b, c, d**). The results of the pedestrian, intraspecific aggressive and natural disturbances all showed a similar pattern. When considering any differences between the time females spent engaging in behaviours before and after these three types of disturbances took place, the only behavioural category which saw a significant difference was resting behaviours. Females spent significantly less time engaged in resting behaviours after any of these three disturbance categories took place, than in the two minutes prior to the disturbance taking place. All other behavioural categories were once again insignificantly different (**Table 3.14**).

Table 3.13 Outcome of GLMM analysis on the factors which affect the duration which females exhibited certain behaviours during lactation with a 10second time lag. The + sign indicates categorical factors included in the model which are deemed to significantly affect the behaviour of an individual. The ΔAICc score give the best model (with the best model scoring a 0). Higher ΔAICc scores indicate models which do a worse job of fitting the data. A ΔAICc score limit of 6 was placed. Factors included in the GLMM were POF aircraft, pedestrian, natural and aggressive disturbance, location, temperature, day in field, daily visitor numbers, distance to fence, maximum sound level, ORQ score, mother-pup distance, pup stage, the number of conspecifics in 5 body lengths, visibility and ID (as a random factor). "NA" refers to factors which did not significantly affect the percentage of the focal (POF) individuals spent in certain behaviours according to the model. A GLMM was run for each behaviour category e.g. pup-check, alert, comfort etc. Negative numbers indicate negative correlations.

	AGG	AIR	TEMP	AVGPEOP	CPRAF	DIF	DisFence	MSOUND	MORQ	MPdIST	NAT	PEOP	PS	TI5BL	VIS	DIF:PS	DF	AICc	ΔAICc	INT
ALERT																				
Model 1					+	0.04											4.00	52.38	0.00	-3.13
Model 2						0.02											3.00	55.11	2.73	-2.11
Model 3																	2.00	55.50	3.12	-1.63
Model 4					+												3.00	55.91	3.53	-2.03
COM																				
Model 1						0.09											3.00	26.43	0.00	-4.74
MOVE																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	10.95	0.00	-4.59
NURSE																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	40.88	0.00	-3.28
MPBEH																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	35.07	0.00	-3.19
THREAT																				
Model 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	16.76	0.00	-4.03
REST																				
Model 1					+	-0.07											4.00	96.37	0.00	2.48
Model 2						-0.05											3.00	100.62	4.25	1.53
VIG																				
Model 1					+	0.05											4.00	57.87	0.00	-3.25
Model 2						0.04											3.00	62.34	4.48	-2.16
PUPC																				
Model 1						0.09											3.00	16.06	0.00	-5.46
Model 2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.00	20.28	4.22	-3.37

Table 3.14 Outcome of Mann Whitney U analysis comparing the duration that females spent engaging in behaviours two minutes prior and two minutes following a disturbance event. Those highlighted in bold are significantly different at the 0.05 level. The type of disturbance is listed in the left hand column.

Disturbance	Behaviour	MEAN (BEFORE)	MEAN (AFTER)	Mann u	P VALUE
Aircraft N=49 FEMALES FOCALS=249					
	PUP-CHECK	2.104	1.815	31803.5	0.9721
	ALERT	9.581	8.931	31518	0.8726
	MOVE	0.685	0.78	31736	0.9889
	COMFORT	3.267	2.467	31535	0.881
	REST	**35.53**	**22.8**	**35573**	**0.01187**
	THREAT	1.361	1.078	32146.5	0.666
	NURSING	1.511	1.254	31648	0.8711
	MOTHER-PUP	2.167	1.551	32070.5	0.7573
People					
	PUP-CHECK	2.538	2.165	31225	0.7123
	ALERT	12.54	11.58	30910.5	0.5728
	MOVE	0.7323	0.9194	30500.5	0.224
	COMFORT	6.924	4.274	31698.5	0.9703
	REST	**46.68**	**28.89**	**37572.5**	**0.000236**
	THREAT	0.8444	0.8106	30898	0.2958
	NURSING	1.566	1.89	30906.5	0.2408
	MOTHER-PUP	2.68	2.078	31518.5	0.8126
Aggression					
	PUP-CHECK	2.636	2.827	31123.5	0.6666
	ALERT	14.93	13.99	31964	0.8891
	MOVE	1.122	1.018	32072.5	0.776
	COMFORT	3.468	2.211	31933	0.8941
	REST	**34.55**	**22.96**	**35224**	**0.0251**
	THREAT	1.674	2.144	30807	0.3507
	NURSING	1.621	1.338	32007	0.7203
	MOTHER-PUP	2.873	1.94	32086.5	0.7308
Natural					
	PUP-CHECK	0.677	1.273	30660	0.3257
	ALERT	6.453	7.006	31184	0.6499
	MOVE	0.436	0.4224	31030.5	0.3232
	COMFORT	1.003	1.658	31855.5	0.9238
	REST	**27.7**	**15.59**	**34615**	**0.0354**
	THREAT	1.084	0.09539	32791.5	0.0726
	NURSING	1.525	0.9005	32252	0.3144
	MOTHER-PUP	1.628	0.9903	31765.5	0.9854

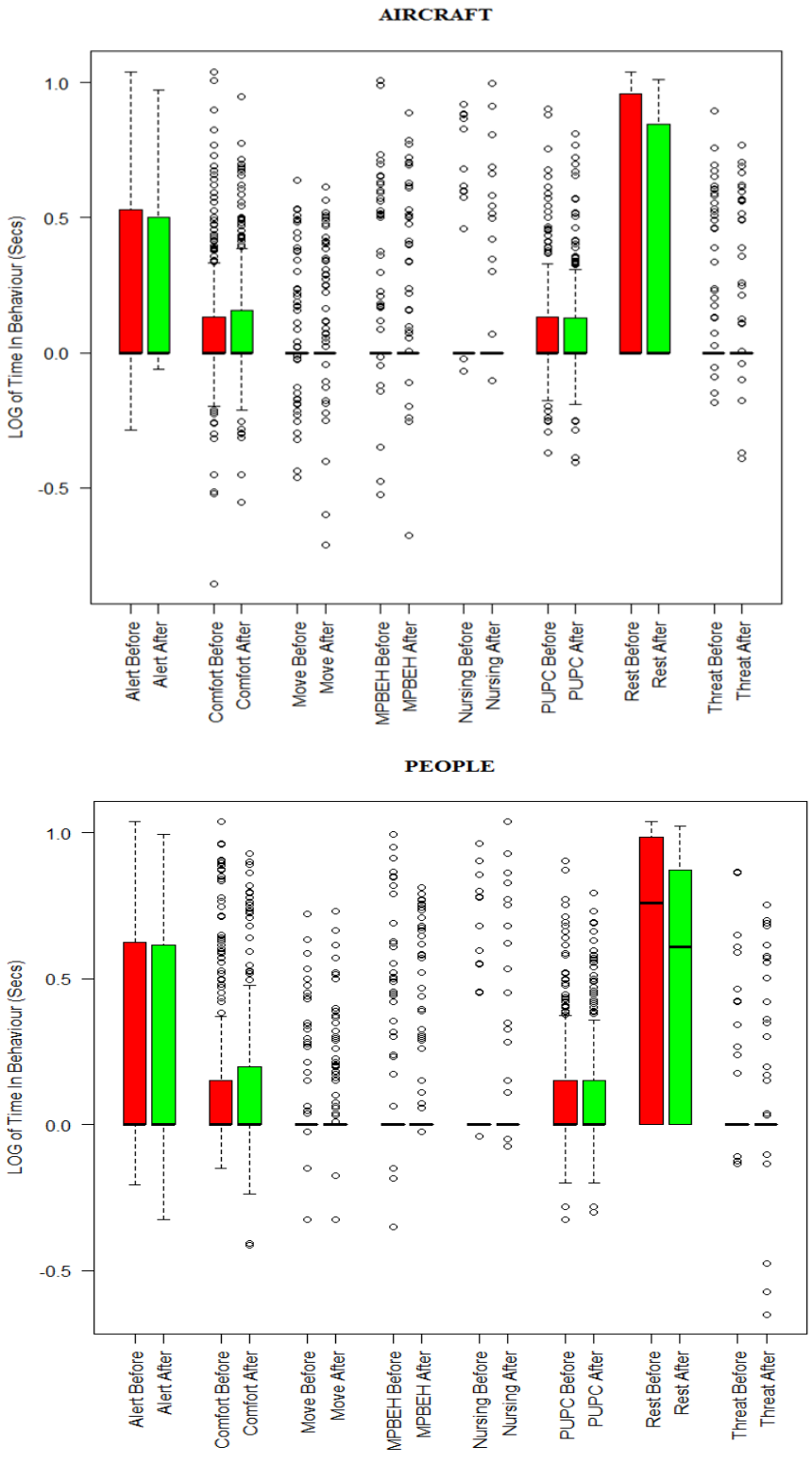

Figure 3.14: a) Aircrfat Disturbance b) Pedestrian disturbance. A comparison of the time spent in certain behaviours two minutes prior (red bars) and two minutes after (green bars) a disturbance event. The symbol ○ represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The log of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data.

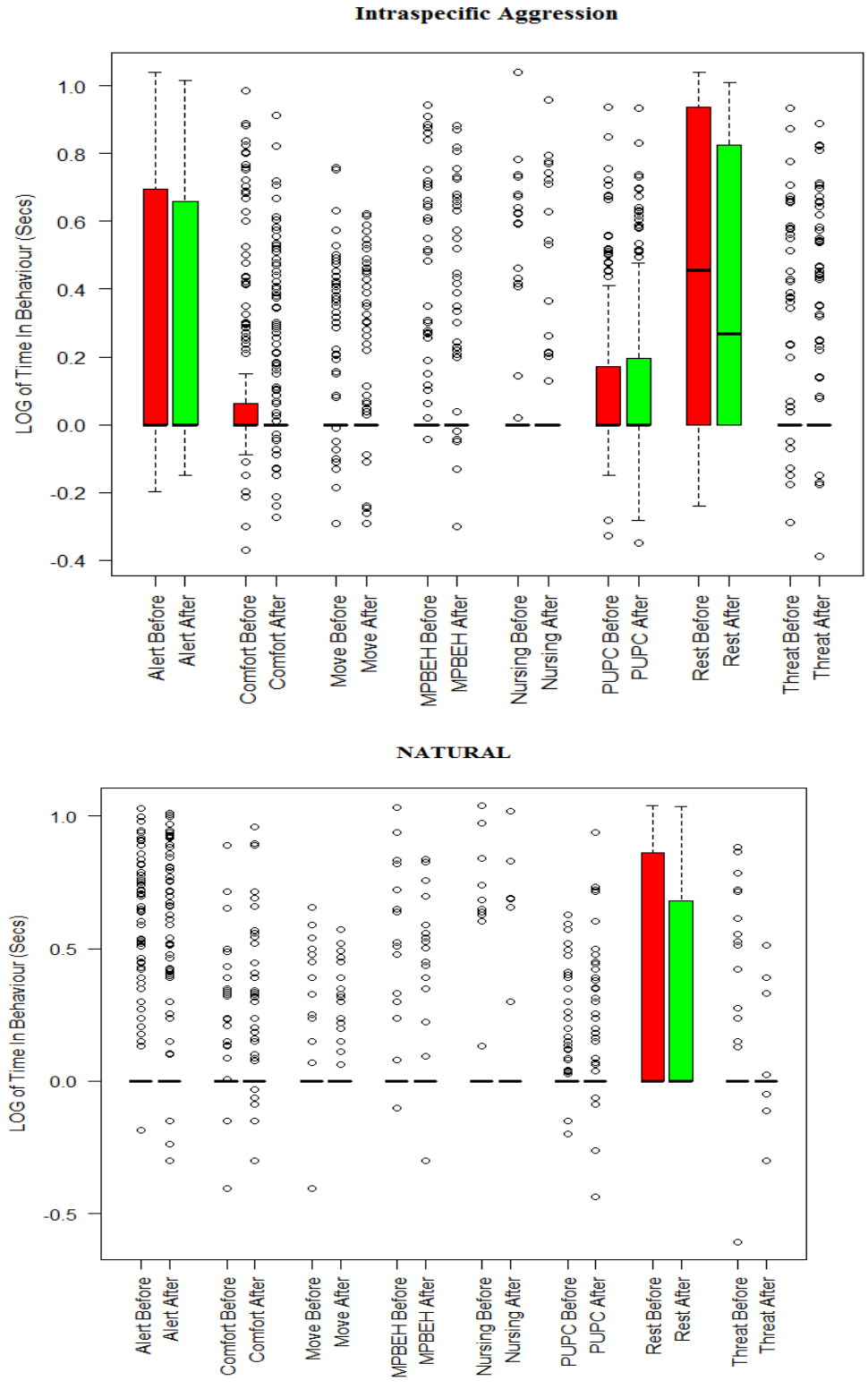

Figure 3.14: c) Intraspecific aggression d) Natural disturbance. A comparison of the time spent in certain behaviours two minutes prior (red bars) and two minutes after (green bars) a disturbance event. The symbol O represents an outlier. The black line within the boxplot represents the median, with the edges of the boxes represesnts the upper and lower quartiles. Cicles outside of the main boxplot represent outliers. The log of behaviour values wereused insteadof normal behavioural perecenatge values in order to create a sensible graphical representation of the data.

4. DISCUSSION

The analyses conducted in this study have provided preliminary evidence in support of anthropogenic disturbance stimuli having a limited effect on female breeding behaviour at the Donna Nook colony. The results of the generalised linear mixed models (GLMM) identified that none of the nine behavioural parameters identified were affected by the occurrence of any of the four disturbance parameters investigated in this study. Furthermore, the behaviours of the females at the site were not affected by visitor demographics, visitor attendance parameters, or any of the sound parameters tested. When considering the immediate effects of disturbance events on the behaviour of females, there was a significant fall in the amount of time females spent resting immediately after a disturbance event (other than when aircraft disturbances were present), when compared to the amount of time a females spent resting in the two minutes prior to a disturbance event. In addition to this, when aircraft disturbances occurred there was a significant decrease in the amount of time females at the two locations spent in threat behaviours when compared to the duration of threat behaviours in the two minutes preceding an aerial disturbance. Individuals in the study exhibited a greater behavioural response to natural disturbance stimuli than to disturbances of anthropogenic origin. Consistent individual differences (CIDs) in vigilance behaviours were noted across disturbance contexts indicating the potential role of habituation and/or selection for behavioural types within and across colonies with differing levels of anthropogenic disturbance.

The study provided some preliminary evidence to suggest that females show a differential maternal response to disturbance stimuli according to the pup's gender. Mothers of male pups tended to have higher alert and pup checking responses to disturbance events when compared to mothers of female pups. In addition to a gender effect at the site, the pupping location also appeared to have some effect on the behaviour of individuals. Females which pupped closer to the RAF base had lower levels of resting behaviour and significantly higher rates of alert, pup checking, vigilance and threatening behaviours than those female which chose to give birth close to the public car park.

4.1. IDENTIFYING MOTHERING STYLES

4.1.1 Differences in maternal time budgets

Referring back to aim 1 outlined in section 1.10.1., when the disturbance context is ignored, the current study suggests that mothers spend more time engaging in pup checking, alert and vigilance behaviours in early lactation (stage 1 and 2) when compared with late lactation (stage 3 and 4). The amount of time that females spent in vigilance behaviours appears to decrease as the pups near independence. In some ways it may be intuitive to note that females show higher levels of vigilance behaviours when rearing younger pups. A younger and more vulnerable pup is less able to respond to dangers posed from conspecifics and other disturbance sources, thus it is vital that females spend longer ensuring that their younger, less mobiles pups are free from danger. It is possible to describe this increased vigilance duration in early lactation as an increase in maternal time investment (Kovacs, 1987; Baker and Baker, 1988).

The duration that females spent engaged in nursing and mother-pup behaviours did not change between early and late lactation when the disturbance context was ignored. This is in agreement with a number of other studies on pinnipeds which have reported no differences in nursing duration throughout the lactation period (Fogden, 1971; Kovacs, 1987). Grey seal mothers must form a strong bond with their pup immediately after birth in order to recognise their pup (Kovacs, 1987). The primary way in which this bond is established is through the pup's unique scent (Fogden, 1971; Insley *et al*., 2003). Interactive behaviours initiated by the mother have been suggested as a form of bonding between the two (Wilson, 1974). Whereas other mammals with parental care groom their offspring to stimulate a bond with the offspring (Weaver and de Waal, 2002; Martin and Reale, 2008), it is possible that grey seal mothers use play interactions between themselves and their pup to maintain the bond between themselves and their pup, and that this is why play is performed equally throughout early and late lactation.

There are difficulties putting findings of the current study into the context of existing literature. Fogden, (1971) produced an in-depth description with visual support for smelling, flippering, presenting and nursing behaviours; however these behaviours are not vastly relevant to the current study on the effects of disturbance. Kovacs, (1987) is slightly more pertinent to the results of this study, providing us with time budgets for

various maternal behaviours on the Isle of May, accounting for nutritional and vigilance behaviours, as well as for solitary behaviours and resting periods. The relevance of the results from Kovacs' study are raised further by the fact that Kovacs, (1987) study was split between three different sites and accounted for differences in behaviour dependent on the to sex of the pup. In comparison to Kovacs study, (1987), mothers at Donna Nook spent, on average, longer alert than those in Kovacs' study (16% of total time compared to 5% on the Isle of May). During the current study, percentage of time spent nursing was lower than that found by Kovacs. Nursing duration remained between 8 and 12% of overall time on the Isle of May, whilst on Donna Nook the average nursing duration was 3.5%. This significantly lower nursing duration at the Donna Nook site may be found as a result of the sampling method conducted at the site. The fact that 30minute focals were employed in this study; along with the infrequency with which nursing behaviours occur between mothers and pups may mean that many nursing bouts were not recorded in the current study.

Twiss *et al.,* (2000) used maternal behavioural categories, similar to those in the current, study, when assessing behaviour on the Isle of May. Similar to the current study, Twiss *et al.,* (2000) used two sites for assessment on the Isle of May: West Rona Beach and Tarbet. The behavioural categories that were used for Twiss *et al.,* (2000) study were: resting, alert, aggression, interacting with pup (PINT including nursing), locomotion or sexual interactions. Data was collected using scan samples at 2 minute intervals over a number of 30 minute periods. Percentage of scans recording each behavioural category was calculated for all 30 minute samples. The data from Twiss *et al*., (2000) are comparable to the results obtained from the current study, as, similar to this study, the 30 minute sample periods neither specifically include nor exclude nursing bouts. However unlike the current study which measures the percentage of the total time in certain behaviours, Twiss *et al*., (2000) study measured the percentage of scans within 30 minutes in which a specific behaviour was noted. Nevertheless, comparisons can be drawn between Twiss *et al.*, (2000) study and the current study by comparing the data of the current study qualitatively to that collected on the Isle of May. The comparison suggests slightly higher levels of mother-pup interaction at the Donna Nook site when compared to the Isle of May. This being said, this conclusion must be made with care as the nature of the data collection method used on the Isle of May was discrete, such that those mother-pup interactions which were of a short duration (for example, play behaviours) may not have been identified by the 2-minute interval scans. Data collection during the current study at Donna Nook

was continuous and so accounted for all mother-pup behaviours within the 30 minute focal.

During the current study, proximity maps identified only a handful of occasions during which a mother was found outside of a 5bl radius of her pup. This is much different to other sites such as the Isle of May and North Rona where females are often noted to have moved great distances from their pup (Kovacs, 1987; Twiss *et al*., 2000; Redman *et al*., 2001). The Isle of May and North Rona sites have a rocky topography, with water available only in isolated pools (Redman *et al*., 2001). In contrast Donna Nook is a beach colony. Upon rain events during the breeding season, water collection occurs in the dips and trenches within the colony. This, in conjunction with the fact that the sediment on which the inner colony forms can be easily dug to expose a cool under-layer, which may allow mothers at the Donna Nook colony access to *in situ* thermoregulation, thus giving them a different behavioural time budget than observed in prior studies of grey seals at the Isle of May or North Rona colony, which may have to spend longer away from their pups for thermoregulatory purposes (Pomeroy *et al.,* 1999; Twiss *et al.,* 2000).

4.2 IDENTIFYING THE EFFECTS THAT ANTHROPOGENIC DISTURBANCES HAVE ON FEMALE BEHAVIOUR

4.2.1 Differences in the maternal time budgets in disturbed and non-disturbed conditions.

It has been suggested that for a number of pinniped species including the grey seal, the disturbances caused by human visitors and other sources of anthropogenic sources at haul out sites can have significant impacts on breeding behaviour (Fogden, 1971; Stevens and Boness, 2003; Engelhard *et al*., 2001). If this is indeed the case for grey seals at Donna Nook, behavioural time budgets should be different between focals in which there were instances of anthropogenic disturbances, and those focals where disturbances were absent.

In answering question 2a outlined in the aims in section 1.10.1., on initial inspection of the data provided by this study using univariate analyses, the presence of humans on a daily basis throughout the breeding period appears to have minimal impact on maternal behaviours. When comparing focals with and without aircraft disturbances, the presence of aircraft disturbance stimuli made no significant differences to any of the

maternal behaviours analysed. Previous studies have suggested that the presence of aircraft disturbances on the site have increased the vigilance response in pinnipeds (Born *et al.*, 1999; Osinga *et al.,* 2012). This was not observed in this study.

When the responses to aircraft disturbances were dissected into aircraft type in order to answer question 2b outlined in the aims, a minimal behavioural response by females was observed. Those focals with non-military aircraft and one rotary helicopters elicited no significant behavioural changes whatsoever when compared to focals in which they were absent. With regards to jet aircraft, the only significant trend was for more threat behaviour in those focals with jets present compared to those where they are absent. When comparing focals with military aircraft and those without, those with had higher levels of pup-checking behaviour and finally, those focals which had no twin rotary helicopters had higher levels of comfort behaviour than those focals in which they were present. In conclusion, the results of this study indicate that aircraft disturbances at the Donna Nook site have very little impact on the behaviour of the breeding females at the site. As seals have a rather poor in air hearing ability (Kastak and Schusterman, 1998), it may be that this limited response to aircraft disturbances may be due to their physiological makeup rather than them simply ignoring the aircraft. In order to establish whether this is indeed the case, more research must be done on establishing the exact hearing ranges of each species of pinniped in both air and sea.

With respect to pedestrian disturbances on the site, the results of the univariate analyses revealed that during focals with visitor disturbances, there was an increase in the duration of pup checking behaviours, indicating that in answer to aim 2a there is some evidence of behavioural responses to pedestrian disturbances by individuals on the colony. Additionally, there was a significant increase in the time females spent nursing and in mother-pup behaviours during focals in which there were no visitor disturbances than when visitor disturbances were present. It was speculated in section 4.1.1 that the outcome of these play behaviours may be homologous to those brought about by licking and grooming in other mammals. Similarly, the mother-pup interaction (MPBEH) behaviour in grey seals might be acting to form and strengthen a bond between mother and pup. It would, therefore appear that the presence of human disturbance is negatively affecting the pups by decreasing the interaction levels with their mothers. This being said it must be considered whether this decrease in maternal interaction is actually harmful to the pup's development, or whether this decrease in maternal attention will increase the exploration levels as described in guinea-pigs and primates, better preparing them for independence (Albers *et al.,* 2000). The behaviour

of colonies of harp seals (*Phoca goenlandica*) in the Gulf of St Lawrence, Canada were compared between those exposed to tourism and those not (Kovacs and Innes, 1990). Non-exposed colony behaviours were used as a baseline for comparison of tourist colonies during and after bouts of visitors. Similar to the results of the current study, Kovacs and Innes, (1990) indicated that during times of human disturbance, mothers spent a reduced amount of time nursing their pup in the few hours after a disturbance occurred. The study concluded that this reduced time nursing did not significantly affect the breeding success of the colony in the year studies. This is once again similar to the results obtained from the Donna Nook colony; although the amount of time individuals spend nursing decreases in the presence of visitors, this reducing in nursing time does not seem to affect the breeding success of the colony as whole, as the number of pups born on the colony has seen a general increase in year upon year, even though the number of visitors to the site has also increased.

The percentage of the focal (POF) females spent performing pup-check behaviours was significantly higher during pedestrian disturbed focals than those where no visitor disturbances occurred. Before discussing the implications of this finding, we must consider the data used to obtain it. Firstly, the data set was particularly skewed, 164 focals contained visitor disturbances and only 84 focals did not have visitor disturbances. However, the results of the focals with pedestrian disturbances consistently had higher durations of pup-checking behaviours than the average value for non-disturbed focals, irrespective of lactation stage or other disturbances which occur in the focal. This would suggest that human disturbances may be provoking an increase in pup directed behaviours in grey seal mothers. Unlike the play behaviours, pup-checks do not, in themselves, increase contact between mother and pup and are therefore unlikely to cause any significant impact on the future behaviour of the offspring, but by increasing the pup-checking, there is a higher likelihood of females spotting potential dangers to the pup, therefore perhaps increasing the likelihood of the pups survival to independence; although this theory was not tested in the current study.

The Donna Nook site has a team of wardens whose job is to patrol the public walkway during the grey seal breeding season. In addition to this, the wardens at Donna Nook have erected two fences between the walkway and the colony in order to increase the safety to both the seals and the visitors. These fences are around a meter in height and so do not provide the colony with any protection from any of the auditory or visual disturbances produced from the visitors. At the weekend it is possible for visitors to cross the fence line and interact directly with the colony; although the occurrence of

this is quite rare. A colony of South American fur seals (*Arctocephalus australis*) in Uruguay were separated from tourists using a similar “countryside type” fence. Cassini *et al,* (2004) compared behaviours on the colony the year before and the year after the fence was introduced. The study noted that irrespective of the fence being present, the louder, or more intrusive human behaviours, created the more intense and negative response from the colony (Cassini *et al*., 2004). However the study did note that the erection of the fence reduced the intensity of the responses to disturbance events, most noticeably a reduction in the amount and duration of aggressive and fleeing behaviours by females at the site. There was also a reduction in behavioural responses to larger groups of visitors. It is possible therefore that the presence of the fence at the Donna Nook site is responsible, at least in part, for the behavioural similarities between focals in which disturbances are absent when compared to those focals where visitor disturbances are present. The results of the analyses conducted in this study seemingly agree with the study by Cassini *et al.*, (2004). There was no correlation with any of the behaviours and any of the visitor attendance parameters or the demographic of visitors. The only visitor demographic which seemed to alter behaviour was the number of photographers present within a 10m radius of the seal from the fence line. There was a trend for more comfort movements when fewer photographers were present and a trend for higher levels of threat behaviour when photographers were present. If we consider photographers as a highly intrusive disturbance, the results of this study are similar to those observed by Cassini *et al.,* (2004) whereby the intense behavioural responses are reserved for the more intrusive visitor behaviours.

With respect to aim four outlined in section 1.10.1., the results of the studies analysis suggests that females who pupped closer to the fence line exhibited higher levels of comfort, pup-checking and vigilance behaviours than those who pupped further from the fence line. In addition to this, females who pupped further from the fence line spent a larger POF engaged in resting behaviours. This result suggests that disturbances caused by visitor behaviour or visitor numbers may be having some impact on the behaviour of individuals. This being said, it cannot be ruled out that some other factor not accounted for in this study may be causing these trends in behaviour. Although visitor numbers fluctuated during the course of each day and peaked during the weekends, there was seldom, if ever, a prolonged period during daylight hours when the footpath was completely clear of humans. This means that, unlike the harp seals of St Lawrence or the elephant seals on Macquarie Island (Engelhard *et al.,* 2002*;* Kovacs and Innes, 1990), the grey seals at Donna Nook did not experience a recovery period in which behavioural changes, triggered by disturbance events, could be reversed.

Consequently it should be expected that disturbance behaviours (decreased maternal attendance, shorter and fewer suckling bouts, increased alertness) will be present consistently at the public site. This increase in disturbance responses could be detrimental to the survival of the pup, by possibly reducing the amount of time a female can spend protecting her pup, focussing more on self-preservation. Clearly this is not the case at Donna Nook, the colony is thriving and the pup numbers are increasing every year (Thompson and Duck, 2010). This being the case, an incomplete picture of the behaviour of individuals may have been obtained as a result of the sampling protocol (see section 4.4.1a for more information), or it may in fact be true that females at Donna Nook have shorter nursing bout durations when compared to other colonies. In order to distinguish whether this is indeed the case, further studies in this area will need to be conducted.

Intriguingly, the study revealed a trend for females to spend longer engaged in alert and vigilance behaviours when there were fewer visitors at the site. This appears contrary to what may be expected, but considering the context of when the fewest visitors are at the site, this may provide an explanation: The days when there are fewest visitors to the site, correlate with the days which are the coolest and experience the highest rainfall. As the grey seals fast whilst ashore, relying solely on the blubber they have built up prior to coming ashore, and must regulate their body temperature while ashore, they may become more active and responsive when it is cooler and after rainfall events as the pools of water which develop at the site after rainfall events may help control thermoregulation. During times of higher rainfall there is an insignificant, but noteworthy trend of increased intraspecific aggressive encounters. The higher movement of individuals seen during these conditions may pose greater threats to the pup from conspecifics, leading to females performing more alert and vigilance behaviours under these conditions. The results of this study therefore suggest that future studies should factor in fine temporal and spatial scale weather when examining disturbance effects on individuals.

In answer to question 2a outlined in section 1.10.1, the GLMMs which incorporated all potentially influencing factors, confirmed that females at the Donna Nook site do not appear to be responding behaviourally to the anthropogenic disturbances occurring at the site. The separate GLMMs run for both the duration and frequency of behaviours in focals reveal that none of the disturbance parameters analysed in this study affects the maternal time budgets of females in the study. The only factors that this study investigated which were deemed to significantly affect the maternal budgets of females

in this study were the day in field and the location of the female (i.e. whether they chose to settle close to the public car park or close to the RAF base). The weather, visitor attendance parameters and the density of conspecifics surrounding the female did not affect the maternal time budgets of females at the site. The stage in lactation that the pups were in affected the time spent in some of the behaviours; namely rest and vigilance behaviours. Females spent more time in vigilance behaviours and less time resting with early staged pups. The results of these models suggest that there are other factors which were not included in this analysis which affect the maternal time budgets of females.

Observations by James, (2013), and some of the results of this current study lend weight to the idea that human disturbances could be placing a pressure on pupping site selection by females at the Donna Nook colony. Another explanation for the similarity in behaviour between the disturbed and undisturbed focals is that individuals on the public site may have become habituated to the disturbance stimuli. Habituation has been defined as "response decrement as a result of repeated stimulation" (quoted from Thompson and Spencer, 1966). Depending on the permanency of the reduction in the response to a disturbance event, habituation could take place either in the few days spent on the beach prior to pupping, or be built up over a number of years due to site fidelity (Cassini *et al.*, 2004; Petel *et al.,* 2008). The occurrence of habituation to human disturbances has been tested in Weddell seals (*Leptonychotes weddellii*) on breeding colonies in the Antarctic (Petel *et al*., 2008). Habituation was found to occur when human visits were made regularly within a short period of time. The total number of seals performing alert behaviours reduced from 67% to 18% by the 10th human visitation in the space of two hours. There was a reduction in the duration of these alert behaviours also. Irregular disturbance over several weeks did not lead to habituation (Petel *et al*., 2008). The habituation theory at Donna Nook is supported by Thompson and Duck, (2010), who argue that the increase in colony size is evidence that human disturbance is not causing a negative impact on seal breeding. As the Donna Nook colony was already an established MOD base and visitor site before the colony first established itself on the beach, it may well be that the seals which chose to breed there may already have some level of tolerance towards anthropogenic disturbance stimuli.

Although the results of this study do suggest that there may be some level of habituation to anthropogenic disturbances exhibited by the seals at the colony, it is important that the study must not conclude that this is definitely the cause of the lack of response to anthropogenic disturbance stimuli. As the study only took note of the

behavioural responses of individuals during the breeding season, one cannot assume that individuals evoke the same limited behavioural responses in periods outside the breeding season. In many cases conclusions of the impact of disturbance events on individuals based on results from the breeding seasons alone would erroneously suggest that human disturbances have a limited effect on individuals within the disturbed population; often misinterpreting the responses of animals as habituation (Bejder *et al.,* 2006). During the breeding season it may well be that females focus solely on breeding behaviours regardless of the anthropogenic disturbances going on around them. For instance Bejder *et al*., (2006) indicated that female New Zealand fur seals (*Arctocephalus forsteri*) responses were stronger both before and after the breeding season, with the strength of the seal responses not persistently waning, thus indicating a lack of habituation outside of the breeding period (Bejder *et al*., 2006). In addition to this, Anderson *et al.,* (2011), Renouf *et al*., (1981) and Kovacs and Innes, (1990) studies on harbour seals (*Phoca vitulina*) all reported that an individual's levels of alertness to approaching disturbers was significantly shorter during breeding season than prior to, and post breeding season, irrespective of the source of the disturbance (Anderson *et al.,* 2011). There is clearly difficulty in demonstrating the process of habituation in wild animals due to the number of other factors which may provide a false indication that habituation has occurred. Determination of habituation in grey seals can be confounded by ontogenetic shifts in behaviour, especially when comparing the breeding season and non-breeding season behaviours. Measuring the physiological responses of individuals as well as the behavioural responses could be a useful approach. Wrongful application of the term habituation can mislead wildlife managers to conclude that anthropogenic activity has benign consequences for wildlife which has the potential to seriously undermine management plans for an area (Bejder *et al*., 2006).

4.2.2. Comparing the responses to anthropogenic disturbances to the responses to natural disturbances

Although responses to anthropogenic disturbance stimuli were the primary focus of this study, the responses of females to natural disturbance events were noted. When comparing the behavioural responses of females to anthropogenic and natural disturbance events in order to answer question 3 set out in the aims, females tend to show a broader range of alterations of behaviour to natural disturbance stimuli than anthropogenic disturbance events, particularly in the form of intraspecific aggressive encounters occurring within a close proximity to the focal female (10m). Those focals

which contained instances of intraspecific aggressive encounters had higher levels of vigilance and threat behaviours than in those focals in which conspecific aggressive encounters did not occur. In addition to this, those focals where aggressive encounters occurred, less time was spent engaged in resting behaviours than in those focals without disturbance. The behavioural responses of individuals to aggressive encounters was similar to those observed in pedestrian disturbances; however there were more behaviours which were affected by aggressive encounters than pedestrian disturbances. In addition to this, the intensity of the response of those behaviours which were affected by both intraspecific aggressive encounters and visitor disturbances was greater in aggressive encounters than in the presence of visitor disturbances. This suggests that females in the study were potentially more threatened and alarmed by aggressive encounters by neighbouring conspecifics than by human visitor presence. This once again could be related to females being habituated to the behaviour of visitors or may be because the behaviour of visitors may be seen as more predictable to the seals than the behaviour of conspecifics.

4.3 POSSIBLE DRIVERS OF MATERNAL BEHAVIOUR IN RESPONSE TO DISTURBANCE EVENTS

4.3.1 Pup Gender

In response to the aim 5 outlined in section: 1.10.1., the current study identified differences in maternal behaviours between mothers rearing male pups and those rearing female pups (Anderson and Fedak, 1987). This corresponds with the findings of a number of studies around Britain, but in contrary to many of those based outside of the UK (Smiseth and Lorentsen, 1995a).Disregarding the disturbance context, mothers of male pups spent significantly more time in threat and vigilance behaviours, and spent less time engaged in resting behaviours than mothers of female pups.

In this study the responses of females to disturbance events were compared between mothers of male and female pups. In the case of aerial disturbances, there was a trend for mothers of male pups to perform more pup-check, alert and vigilance behaviours than mothers of female pups when aerial disturbances were present. Smiseth and Lorentsen, (1995a) observed a lack of effect of pup gender on grey seal maternal behaviour or investment while studying a colony on Froan, Norway; with the exception that mothers with male pups spent more time in ‘visual contact’ with their pups.

Interestingly, the results from the current study also suggest a trend towards mothers with male pups spending, on average spending a larger proportion of their time performing alert, pup-checking and vigilance behaviours. When visitor disturbances were present, mothers of male pups once gain spent significantly longer engaged in vigilance and threat behaviours and spent significantly less time resting than mothers of female pups. A similar response was also observed when intraspecific aggressive encounters occurred within the focal. It is generally expected that mothers with male pups should show higher levels of maternal investment than those with female pups. This is due to the fact that larger adult males tend to have a higher reproductive success than their smaller male counterparts, whereas all females have similar reproductive success irrespective of their body (Amos *et al.,* 1993; Twiss *et al*., 1998; Lidgard *et al.,* 2005).

No differences in the level of nursing or mother-pup interactions between the sexes were noted in this study. There have been a number of suggestions as to why this difference in maternal investment in nursing and mother-pup interactions may not occur between the two pup sexes. Studies on different pinniped species have suggested that maternal investment does not bias one pup gender over the other due to the fact that weaning weight has been found to influence both pup genders' survival rates during the first year (Smiseth and Lorentsen, 1995a; Hall *et al*., 2001). Anderson and Fedak, (1987) carried out behavioural observations on the island of North Rona. Though their results suggested greater weight gain by male pups than female pups, their behavioural observations suggested no significant differences in nursing rates between male and female pups. Mothers of male pups also lost more weight those mothers of female pups in the study by Fedak, (1987). The results of this study do not support the notion of a difference in social interaction from the mothers dependent on the pup's gender; the duration of mother-pup behaviours was similar in mothers of female and male pups. A further reason as to why females may not bias the amount of a nursing a pup receives based on gender may be due to the fact that the female is responsible for placing a limit on expenditure during rearing, with relation to the amount of time and effort she will invest in nursing (Pomeroy *et al*., 1999), but possibly extending to other interactions such as play and other mother-pup interactions. Female grey seals have an inbuilt limit to the proportion of their stored resources they are willing to expend whilst ashore (usually around 46.5% of the maternal postpartum mass, Pomeroy *et al*., 1999). Past this limit, they may begin to risk their own reproductive success in the future by depleting their limited resources (Pomeroy *et al*., 1999).

4.3.2 Location

It has been suggested that, the location that an individual settles and gives birth on a colony can impact upon both the levels of disturbance caused by human visitors and the way in which individuals react to disturbance events (Stevens and Boness, 2003; Engelhard *et al*., 2001). If this is indeed the case for grey seals at Donna Nook, the behavioural time budgets for the females in the study should be different between those females which reared their pup close to the public car park and those which gave birth near to the RAF base. Both sites were located on a public footpath which is open to visitors and receives several thousand visitors per week throughout the breeding season. Many of the visitors to the site do tend to stay close to the public car park side of the walkway. The RAF site next to the public site is owned by the Ministry of Defence and is cordoned off to the public. This site was not analysed in this study.

Addressing question 4 outlined in section 1.10.1., irrespective of the disturbance context data suggested that those mothers who reared their pups close to the RAF base spent significantly more time engaged in alert, vigilance and threat behaviours than those females located close to the public car park. When disturbance context was taken into consideration, during focals in which aerial disturbances were present females at the RAF site spent more time in alert and vigilance behaviours and less time in resting behaviours than females located close to the public car park. In addition to this, in the presence of pedestrian disturbances, females near to the RAF site spent significantly more time engaged in alert behaviours than females close to the public car park. Although there was no difference in the responses of females to conspecific aggression at the two sites, there were some minimal differences between the responses of females to natural disturbances at the two sites. Females near to the RAF base spent significantly longer engaged in alert and active movement behaviours and less time engaged in comfort movements when compared with the females at the car park site during focals with natural disturbances present.

The percentage of the focal (POF) females spent performing vigilance behaviours was significantly higher at the RAF site during disturbance events. Before discussing the implications of this finding, one must consider the data used to obtain it. Firstly, the data set was particularly distorted, out of the 249 focals, aircraft disturbances were noted in 123 of them. Within this sample only 30 of the focals were recorded at the site close to the public car park, with the other 93 being taken from those females located close to the RAF base. The distribution of these videos with respect to the time in

season also indicated that the majority of the females located at the RAF base were followed in early and late in the breeding season, when the male: female ratios were at their highest at the colony and when visitor numbers to the site were at their lowest. The importance of this distribution is clear from looking at the results of the analysis in which the POF spent performing alert and vigilance behaviours was significantly higher at the RAF site, not only when anthropogenic disturbances occurred but also when the disturbance context is disregarded. This being said, due to the large sample size of focals in the study, it is possible to suggest that that there may be an element of difference between the two sites. This being that the females located at the RAF base have higher levels of vigilance behaviour in focals where aircraft disturbances were present compared to those females located at the site closer to the public car park. The reasons for this increase in vigilance may be due to the fact that the seals close to the RAF site are in a closer proximity to the central aircraft operations/ landing pad than the seals which lie closer to the public car park. Furthermore, this increase in vigilance could be as a result of another confounding factor such as differences in topography, personalities of females or the quantity/ intensity of others forms of disturbances at the two locations.

There was no difference in the level of nursing, threat or mother-pup behaviours in the presence of any of the disturbances between the two sites. It would, therefore, appear that the location that a female raises their pup on a colony in the presence of human disturbance does not alter the interaction level between pups and their mothers. As the level of nursing and mother-pup interactions have in previous studies been shown to impact upon the survival rate of a pup to independence (Anderson and Fedak, 1987; Bowen *et al.,* 2006), this study shows that where a female chooses to give birth at the public site, should not in itself affect the probability of survival of the pup.

It is possible that the presence of disturbances at the public site, combined with the accessibility of the nearby, relatively undisturbed RAF site, places a selective pressure on individuals hauling out onto the colony with relation to pupping site. If an individual had a low tolerance to disturbance; pupping and breeding attempts on the public stretch of the colony may be relatively unsuccessful. Success may increase simply by selecting a pupping spot on the less disturbed RAF site. Those individuals with a higher tolerance towards disturbance may find adequate pupping and breeding success on the public site. This would explain the similarities in maternal behaviour between the two sites in this study and would explain results observed in prior studies conducted at the Donna Nook site (James, 2013). James, (2013) noted that individuals located at the

RAF site at Donna Nook were exposed to a digger driving through the colony every morning and evening. The female's responses to this digger were still extreme to the digger no matter how long the individual had been on the colony; with many individuals showing extended alert and pup-check behaviours throughout lactation. In the same year a digger was only once driven directly through the colony on the public site. Despite having little prior experience of this disturbance, this public site colony remained restful, with few if any alert behaviours (James, 2013). Although not quantified in this current study, from personal observations at both the RAF and public site, this study also noted a difference in the female's responses to the tractor. The females at the RAF base were very nervous and unsettled when the tractor moved through the site. In comparison, although rare in occurrence, when the tractor did pass through a part of the public site, the females remained fairly relaxed with very little behavioural response to the presence of the tractor.

4.3.3 Consistent individual differences in maternal behaviour both across and within disturbance contexts

In answer to the query as to whether individuals at the site show consistent individuals differences in behaviour, individuals in the study exhibited consistent individual differences (CIDs) in alert, resting and pup checking behaviours, in both early and late lactation. These CIDs are apparent across disturbance contexts for some behaviours (i.e. alert and vigilance behaviours), but occur solely within disturbance contexts for other behaviours analysed in the study. Some of the behaviours identified in this study were not found to be repeatable across disturbance contexts, suggesting that these behaviours may be performed at a similar rate in all seal mothers, or may be situation dependent. However, given the small values for average POF spent nursing, threatening and mother-pup interaction (MPBEH) behaviours, and the infrequent occurrence of these two behaviours, 30 minute focal videos may have been unsuitable for identifying how disturbances may have affected the occurrence and intensity of these behaviours. This unsuitability is further discussed in section 4.4.1a.

In agreement with the results obtained by Twiss *et al.,* (2012) the results of this current study have identified CIDs in pup checking behaviours (Twiss *et al*., 2011; Twiss *et al*., 2012), both within and across disturbance contexts. Twiss *et al.,* (2012) compared consistency in pup checking behaviour across situations (as defined by Sih *et al*., 2004; given in) in the North Rona colony. During 2009 and 2010, individually identifiable females were exposed to a novel aural stimuli; a remote control vehicle (RCV) playing

a ‘wolf’ call. Twiss *et al.,* (2012) study showed that CIDs in pup-checking behaviour were present in both a relaxed and disturbed situations. However, unlike the results of this current study, Twiss *et al.,* (2012) found no CID in pup-check behaviour across both disturbed and relaxed situations (Twiss *et al*., 2012). This suggests that mothers at the Donna Nook public site may have a more proactive personality, when compared with the females at the North Rona colony. Proactive mothers in this study respond less to environmental change and perform pup-checks at a fairly constant rate irrespective of situation. This is similar to the response exhibited by the females at the Donna Nook site where behaviours are consistent across disturbance contexts whereas the females at North Rona are more reactive, responding behaviourally to the presence of the disturbance stimuli by altering their pup-check rate according to the situation (Twiss *et al*., 2011; Twiss *et al.,* 2012). This could suggest that the public site at Donna Nook may be selecting for proactive type females, which show a similar response in disturbed and non-disturbed conditions; with individuals which convey a more reactive phenotype, removing themselves from the colony. To test whether this is indeed the case, greater comparisons of female breeding behaviour need to be made between the RAF and public sites at the Donna Nook colony.

Providing the colony at Donna Nook is similar to that on North Rona; with female behavioural types spanning across the proactive-reactive axis, the presence of any disturbance or aggression during the focal would affect pup-checking rates differentially, depending on whether the female had a proactive or reactive behavioural type. This reasoning does not explain the positive identification of CIDs in behaviour across disturbance contexts during the current study. It may be that individuals with a proactive type may differ from each other in how they react to things; but within themselves show similar reactions across situations/contexts (i.e. not flexible). Some proactive individuals may have high vigilance no matter what the disturbance context, whereas others may have low vigilance levels.

The result of this current study, along with the results obtained prior studies on grey seals (e.g. Twiss *et al.,* 2012; James, 2013) indicate that CIDs in vigilance type behaviours are common in grey seal colonies around the UK. There are number of reasons as to why CIDs may be found in vigilance behaviours, such as: for pup protection, self-defence or to explore their immediate surroundings. As vigilance behaviours appear to be a definite CID in seals (and other spp. too), CIDs in vigilance responses need to be considered in studies where vigilance type behaviours are used as a measure of response to disturbance. In terms of this study, CIDs in vigilance responses are important as they shows that vigilance tendencies are individualistic and

emphasise a need for individual level modelling approaches in order to study vigilance responses to disturbance (i.e. GLMMs).

CIDs were not identified in time spent nursing across disturbance contexts. As mentioned previously, no measure of milk transfer efficiency or milk content were collected during this study and so it cannot be concluded that mothers expended similar nursing resources to one another. It is possible that although mothers spent a similar amount of time nursing both across and within disturbance contexts, some mothers may have been less efficient at transferring milk to their pup. Mellish *et al.,* (1999) identified variation between mothers in milk content with respect to fat and protein. The fact that females in this study spent a similar amount of time engaged in nursing behaviours, irrespective of this probable variation in milk content may suggest that pups may have differential survival rates dependent on the quality of the milk produced by the female (Pomeroy *et al.*1999); although for this to be confirmed further studies looking into the efficiency of milk transfer at the Donna Nook colony needs to be carried out. As nursing rates were similar in disturbed and non-disturbed conditions, it may be suggested that disturbance events at the Donna Nook site do not affect behaviours which affect the survival probability of the pup, namely nursing and mother-pup interactions. However, as nursing behaviours were highly infrequent in this study, further analysis into whether this is indeed the case, needs to be carried out.

CIDs in threat and active movement duration were not found in this study. In the case of threat duration, this is likely due to the high intra-individual variation in duration of each bout of threat behaviour. Mothers were highly variable in the length of time each bout of threatening behaviour lasted. It is possible that these two behaviours are entirely responsive to changes in the environmental context and may fluctuate depending on the situation.

Consistent individual differences (CIDs) in behaviour were measured from early to late lactation and both across and within disturbance contexts. When referring to the CIDs which included both early and late lactation and disturbed and non-disturbed conditions; the females at Donna Nook showed CIDs in the amount of time spent in vigilance (pup-checking, alert and vigilance), resting and comfort movements. CIDs were not found for nursing behaviours or mother-pup interactions. Similarly, mother-offspring relationship quality (MORQ) index values calculated from focal videos were found not to be consistent across disturbance contexts and lactation stages implying that those mothers that show their pups greater affiliation, do not do so consistently

from early lactation to late lactation and between disturbed and non-disturbed conditions (disturbed conditions being those with aircraft disturbances). During periods of disturbance, CIDs were observed in all three vigilance parameters across lactation stages. In non-disturbed conditions, CIDs were present across lactation stages in pup-checking and comfort behaviours.

By considering pupping dates, from the work of prior studies on grey seal breeding (Pomeroy *et al.,* 1999), mother-offspring relationship quality (MORQ) scores can be compared to the possible age and experience of the female. Anderson and Fedak, (1987) and Pomeroy *et al.,* (1999) both identified a tendency for larger females to pup earlier in the season. The body mass of female grey seals is also known to increase with age (Bowen *et al.,* 2006). With this information it may be postulated that older mothers pup earlier in the season than younger ones. Addressing questions 7a and 7b outlined in section 1.10.1, for mothers in this study, there seems to be no correlation between relative pupping date (estimated from date at which pup was recorded as stage 2) and MORQ values. Mothers with more affiliative relationships did not check their pup significantly more or less than those with more rejective relationships; however more affiliative mothers did tend to stay in closer proximity to their pup. In addition to this there was no difference in MORQ scores between early and late lactation or between disturbed and non-disturbed conditions (for all four disturbance categories). Thus affiliative mothers are not necessarily more protective of their offspring in disturbed conditions. In order to assess whether the effects of these affiliative behaviours on the offspring are positive or negative, data would need to be collected from the pup itself. Suggestions for this are made in the extensions section 4.4.2.

The presence of CIDs in some of the behaviours across disturbance contexts and lactation stages throughout a single breeding season is preliminary evidence for the existence of mothering styles in grey seals, irrespective of the disturbance context. This lends weight to previous studies, supporting evidence of CIDs in maternal behaviour (Twiss *et al.,* 2012; James, 2013). This evidence is strengthened by CIDs found during in-field pup-checking in previous studies (James, 2013), as well as in pup-check counts from previous literature (Twiss *et al*., 2011; Twiss *et al*., 2012). Although CIDs were found in many of the behaviours, the inconsistency in MORQ index values calculated from focal videos suggests that mothers may alter their affiliative response to pups dependent on the environmental situation. MORQ encompasses a wide range of behaviours and as of yet it is unknown what these behaviours function as; some are

obviously affiliative or rejective, but others such as resting and threat behaviours are more unclear. It is therefore possible that another study could select a whole different subset of behaviours to go into the MORQ calculations and this is a problem for comparing MORQ scores. It is therefore vital that future research can look into an agreement on the behaviours which should be classified as affiliative, and those behaviours which should be classified as rejective. Further data would be required to confirm the existence of CIDs mothering styles both within and across disturbance context, especially in the context of nursing and mother-pup interactions, which occur infrequently in the field. For a further discussion on the possible extensions to this study please see section 4.5.2.

4.3.4 The immediate responses of females to disturbance events

Not only did this study look at the effects of disturbance in terms of the whole focal video, it also examined the immediate behavioural responses of grey seals to disturbance events. In Tracey and Fleming, 2007 showed that once a disturbance has disappeared from the view of a focal individual, the behaviour of an individual will return to normal within a very short space of time (usually in the first couple of minutes) (Tracey and Fleming, 2007). In answer to question 8 outlined in the aims in section 1.10.1., analysis using a 10 second time lag indicated that once again no disturbance sources had any effect on the behavioural time budgets of females. Likewise, examination of the duration of behaviours in the two minutes following a disturbance event relative to the two minutes preceding showed that the only behaviour to be significantly affected by all four disturbance categories was resting behaviour. For all four disturbance parameters, the duration of resting behaviour in the two minutes following a disturbance event was significantly lower than in the two minutes preceding a disturbance event. None of the other behavioural categories showed any significant change before or after a disturbance event, suggesting that a disturbance event does not significantly alter the maternal time budgets of breeding seals immediately after disturbance events. So in conclusion the results of this study indicate although there is a significant decrease in resting behaviour after a disturbance event, individuals do not shift to a particular alternative behaviour (e.g. alert) and so simply become more active generally in the two minutes after a disturbance event. This may be as a result of several processes, such as: individual differences (CIDs) in behaviour and individual differences in reactivity patterns.

Kovacs and Innes, (1990) indicated that during times of human disturbance, mothers spent a reduced amount of time engaged in resting behaviours, with an increase in the time spent alert. The study by Kovacs and Innes, (1990) also indicated a change in pup behaviour after tourist disturbances, with them becoming more agonistic and less restful. However, these effects disappeared quickly after the disturbance was removed, becoming comparable to the baseline behaviours (Kovacs and Innes, 1990). Other studies have been able to compare the behaviour of the same individuals before, during and after the presence of humans. Engelhard *et al.,* (2002) focussed on a colony of southern elephant seals on Macquarie Island. The colony was exposed to periods of human disturbance, both in the form of tourists and other researchers. Whilst humans were present, both the rate of maternal calls and alert behaviours amongst females increased significantly. However, these rates reduced to normal quickly after the disturbance was removed (Engelhard *et al*., 2002). This somewhat contradicts the results of this current study which indicated that there were very minimal immediate responses to any of the anthropogenic disturbance events which occurred at the Donna Nook site. The reasons for this are numerous. Firstly, as mentioned previously the individuals at the public site could be selected for being tolerant towards disturbances. Those individuals which are less tolerant to disturbances may have less successful pupping attempts at the public site. The success of these less tolerant individuals may increase simply by selecting pupping sites on the RAF site where tourists are prohibited from entering. Those individuals with a higher tolerance towards disturbance may find maximum pupping and breeding success on the public site.

The results of this study lends weight to the idea that human disturbances are placing a pressure on the pupping site selection by females at the Donna Nook colony as the lack of maternal responses to disturbance events was evident in nearly all of the females at the public site. Another explanation which has been touched upon in section 4.2.1 is that the similarity in the immediate behavioural responses of females to disturbance events may be due to the fact that individuals on the public site may have become habituated to the disturbance stimuli. The study has found that there seems to be an immediate reduction in resting behaviour and an increase in general activity, not specific behaviours; but these responses are transient; and over longer time scales (30 min focal) activity budgets are not influenced by anthropogenic activities; in fact they are more influenced by conspecific activity. The minimal behavioural responses to disturbance events may suggest the occurrence of habituation to these anthropogenic disturbance stimuli at the Donna Nook site (Thompson and Spencer, 1966).

4.4 LIMITATIONS AND POSSIBLE EXTENSIONS TO THE CURRENT STUDY.

4.4.1 Limitations

4.4.1a Data collection

The first and perhaps most obvious limitation of the study in terms of data collection comes from the fact that data collection was limited to one breeding season. This has prevented the understanding how disturbances effect seals over a number of breeding seasons and prevents the study from fully establishing whether habituation at the site has indeed occurred. However, this study provides preliminary evidence of the fact that during the breeding season, anthropogenic disturbance events seem to have minimal impact on the breeding behaviour of female grey seals at Donna Nook. This leads a clear path for future research.

A second limitation was that data collection protocols were limited to observations. As the site is open to tourists, direct interaction with the seals on the site is not permitted. This means that the weights of both the female and pup at the start and end of lactation were not collected. Previous research relies heavily on these physical measurements to determine the maternal expenditure of females and the importance of this on the development of the pup (Pomeroy *et al.,* 1999). These measurements would have been particularly pertinent to this study as they would have permitted the analysis of the impacts that disturbances have on the weaning weight of pups; as this has previously been link to survival of offspring within pinnipeds (Thompson and Duck, 2010).

Similarly, as research at the Donna Nook site has been fairly limited in the past, with only a handful of other studies known to have taken place there in the last ten years, age and experience data of the females in the study was not available. Further to this, the observational approach prevented a complete record of pup genders from being obtained; as a result, just over half of the females in the study had pups of a known gender as gender identification was not always possible. As a result of the aforementioned reasons, links between maternal behaviour in response to disturbance events to the prior experience of mothers and impacts on pup survival can only be

postulated at this stage as comparisons cannot be formed conclusively with previous literature of studies which were undertaken at the site.

A further limitation of the data collection method was that the nature of the focal videos places limitations on the calculation of duration of behaviours. During a 30 minute focal, an increased time spent on one of the behavioural categories, in response to a disturbance event decreases the time available for others. Certain behaviours, in particular nursing and threat behaviours occur infrequently throughout the day and are often in response to an environmental cue or a cue from a conspecific (Ross, 2012). A single 30 minute video focal is therefore unlikely to provide a true reflection of the time a female spends on both nursing and threat behaviours during the day. An enhancement of this method would be to manually record the frequency and duration of nursing and threat bouts during the day or replace the use of focal videos with short-interval scan samples throughout the day, in order to maximise the daily representation of behaviours by an individual instead of relying on one thirty minute period. The method employed in this study required data to be collected from 6-8 individuals per day. These individuals were all in close proximity to one another. For this reason, if a scan sampling method were adopted in the future, it would be possible to record the behaviour from all individuals using 1 minute scan samples. This would ensure a short enough interval that behaviours lasting only a few seconds (such as alert and pup-check) could still identified, but would provide an opportunity to spread data collection throughout the day, increasing the likelihood of capturing rare behaviours such as nursing or aggressive encounters. This being said, the method used in this current study provided enough data for preliminary investigation into the effects that disturbances have on the behaviour of female grey seals and provided some evidence in support of the fact that pup gender and female location may affect a females responses to a disturbance event. In addition this, the protocol provided a good method of measuring behaviours such as vigilance which, have in prior studies been deemed to be significant in terms of responses to disturbance events.

Finally, previous research has alluded to the fact that it is not only the amplitude of auditory disturbance events but also the frequency of these disturbances which can affect individual behaviour (Albers *et al.,* 2000). This study initially set out to collect both frequency and amplitude data using an on-board sound microphone. However, in the field the on board microphone failed; the data was un-usable due to the strong winds at the site. This meant that the study had to rely solely on amplitude data from a hand held sound meter in the field. In addition to this it would have been informative to

place the planes into altitude levels in order to compare amplitude of sounds with the planes altitude.

4.4.1ai Storm Surge on the 5th December 2013

The storm surge on December 5th 2013 caused a sudden and unexpected disturbance to the colony across the Donna Nook colony as well as much of the East coast of England. As the tidal surge was much later in the season than the tidal surge experienced in November 2011, many of the pups had already reached independence and were leaving the colony for the sea (personal observation). However a number of mother-pup pairs were still present at the colony and many of these pairs were separated; in some cases permanently; and a number of pups were seen without a mother in the days following the tidal surge. The day before the tidal surge hit the wardens cut down the fence line allowing the seals to retreat to the surrounding higher ground. Unlike the 2011 tidal surge, only a handful of pups died as a result of the tidal surge and the majority of abandoned pups were taken in by Mablethorpe seal sanctuary. Prior to the tidal surge occurring six females in the study were still on the colony. After the tidal surge hit only four of these females could be located, and one did not have their pup. The new placement of these females made them impossible to film and so the decision was made to stop the field season slightly earlier than planned. This was unfortunate as previous research gathered from before and after the storm surge in 2011 revealed that females significantly changed their behaviour after the storm surge event (James, 2013). James', (2013) study on grey seals at Donna Nook revealed that the time that females spent engaged in alert behaviours increased after the storm surge. The high variability within females in duration for each occurrence of alert behaviours in the study by James, (2013) suggests that alert duration was responsive to the environment disturbance stimuli. In addition to this, the study by James, (2013) revealed that there was a very clear increase in the time that females spent performing pup-check behaviours. In field pup-check rates showed a significant increase in response to the sudden disturbance.

4.4.1b Study Site

The Donna Nook inner colony is composed of two parts; one part of the colony is located on a part of the beach accessible to the public, and the other is located adjacently to this site on an active MOD base, which is off limits to tourists. This current study solely focussed on those seals located on the stretch of beach which was

accessible to tourists. This posed a number of limitations to the study. Previous studies identifying the impacts of visitor disturbance on maternal behaviour and pup development have been able to compare the same individuals before, during, and after the disturbance (Kovacs and Innes, 1990; Engelhard *et al.,* 2002). For this to be possible an assured period of time without human disturbance is required; something which was never possible at the Donna Nook site. Being a public footpath, and an area of high tourist volume, particularly in the daylight hours when this study took place, there was almost always human presence from dawn until dusk during the breeding season along the stretch of the colony accessible to the general public. This meant that comparisons of an individual female's behavioural in the presence and absence of some of the disturbance sources were difficult to carry out.

The topography of the land also provided some limitations to the study. The landscape close to the fence line at the public site is dotted with sandbanks and trenches which provide hiding places for the seals. During some of the focals females were lost from view for a prolonged period, often meaning the focal was unusable. In other cases, females which should have had a focal on a particular day could not be found as they were hidden behind the sandbanks. Due to the fact that the site is located on an active military base, tourists and researchers could not go onto the beach during the week days as military exercises took place above the beach. As a result of this, when females disappeared from view, movement by the researcher could not be undertaken to find them and restart observations. Furthermore due to the lack of movement permitted by the researcher, if the female changed their orientation away from the camera, subtle behavioural responses to disturbance events such as pup glances and alert behaviours would go unnoticed. In order to establish how much of these subtle behaviours are overlooked in videos, future studies should look to using more in-depth in field techniques to log and record behaviours which take place, noting the surrounding context of the focal individual. In addition to the topographical issues of the site, the exposed nature of the beach site at Donna Nook meant that wind interference was often a problem with recoding auditory data. The high wind levels recorded at the site meant that the on-board sound microphone data was unusable and the hand held sound meter often could not pick up noises above the wind.

4.4.1c Data Extraction

The major limitation in extracting and analysing the data collected from the field was discerning the more subtle behaviours from the videos. Pup-checks and alert

behaviours are often quick and sometimes subtle movements. To identify a pup-check the exact location of the pup must be known, which is not always possible to discern from a video recording. In addition to this, the limited field of vision and poorer sense of depth provided by the recordings makes discerning what indeed the females are responding to in the video difficult. Furthermore the orientation of females in the video may mean that some of the more subtle behaviours performed by females such as alert with head down or pup glances may not be picked up in the analysis of the videos if the female is facing away from the recorder. To increase the reliability of the data obtained from the visual studies, wider field angles from the camera should be obtained in order to ensure that the pup and female are in the video where at all possible. Where this is not possible a continuous note of the pup's position should be made in relation to the mother. This would ensure correct identification of pup checking behaviour from the video playback.

Finally, it must be accepted that there may be some lack of clarity in some of the behaviours made from the video recordings. Some of the behaviours studied such as alerts and pup-checks are quick and sometimes subtle movements, for instance to identify a pup-check behaviour, the exact location of the pup must be known. This study tried to compensate for missing subtle behavioural responses by later grouping behaviours into broader categories of behaviour. If this study were to be repeated, it would be far easier to determine the rate of pup-checks using in-field count due to the fact that it is difficult to fully distinguish pup checking from alert behaviours in the videos, as some amount of context of the surrounding environment of the focal individual is missing.

4.4.1d Using the behavioural responses of individuals to measure the effects of disturbance

Investigations of anthropogenic disturbances must often aim to produce time-sensitive reports under deteriorating environmental conditions (Bejder *et al*., 2006). Owing to inadequate time and funding, studies often rely on short-term, behavioural measures (Lacy and Martins, 2003; Bejder *et al*., 2006). Little is known, however, about the suitability of short-term using measures of behaviour as indicators of the impacts of disturbance on individuals at an effected site (Bejder *et al*., 2006). It is seldom known if any changes in behaviour may influence reproduction, survival or population size (Gill *et al*., 2001; Beale and Monaghan, 2004; Bejder *et al*., 2006). In some studies it has been noted that pedestrian approaches can cause physiological responses in

individuals in the absence of noticeable overt behavioural responses (Antarctic Treaty consultative Meeting, 2008). For example, bighorn sheep (*Ovis canadensis*) and white-tailed deer (*Odocoileus virginianus*) exposed to land vehicle traffic have exhibited heightened heart rates and increased levels of certain hormones, but showed no observable behavioural changes (MacArthur *et al*., 1982; Moen *et al*., 1982). In some cases, behavioural and physiological responses are only detectable over a short time period after a disturbance event, and due to this short response time their biological significance is difficult to discern (Antarctic Treaty Consultative Meeting, 2008).

Behavioural change is often considered the most sensitive measure of the effects of human disturbance on animals (Carney and Sydeman, 1999; Beale and Monaghan, 2004). However, if behavioural responsiveness to disturbance events is positively correlated with an individual's body condition, this may be an inappropriate index to use a measure of effect, as individuals showing the smallest responses may in fact be those with most to lose from changing their behaviour, and so in most need of help (Beale and Monaghan, 2004). Gill *et al*., (2001) argued that when animals have a lot of options when in good condition, they may be more likely to change their behaviour in response to a disturbance than when they are more constrained by current requirements (Beale and Monaghan, 2004). Therefore it cannot be assumed that the most responsive animals are the most vulnerable. Anthropogenic disturbances have the potential to influence many components of a species' behaviour and physiology (Ciuti *et al*., 2012). Repeated disturbance events can initiate cumulative effects on an individual's energetic budget, which have the potential to cause long-term negative effects on their survival and reproductive rates (Christiansen *et al*., 2013). Even subtle effects of anthropogenic disturbance on physiological parameters, such as increased heart rate or stress hormone levels may reduce an individual's fitness level (Frid and Dill, 2002; Ellenberg *et al*., 2006). For example, in many species, the mere presence of humans is associated with significantly increased baseline glucocorticoid levels (Homan *et al*., 2003; Mullner *et al*., 2004). Short-term stress responses are thought to be beneficial in aiding individuals handle disturbance events effectively (Ellenberg *et al*., 2007).

Alternative measures such as measurement of stress levels (Nimon *et al*., 1996; Fowler, 1999) or methods involving measurements of resource use (Gill *et al*., 1996) are needed to allow a more holistic assessment of the effects that disturbances have on individuals. For example, when faced with a disturbance at a an area with a high value of feeding resources, individuals in good condition may be more capable of

bearing the costs of suspending their feeding and moving to other areas than individuals in poor condition, for whom continuing feeding is a high priority (Gill *et al*., 2001; Dyck and Baydack, 2004; Ellenberg *et al*., 2013). Therefore in this instance, individuals in good condition will exhibit a more marked behavioural response to disturbance events, whereas individuals in poorer condition may have no option but to continue feeding for as long as possible despite internal stress due to a disturbance event. In a study by Gill *et al*., (2001) waterfowl species (*Anas spp.*) in sites provisioned with food were more likely to fly away when faced with a disturbance: they were in better condition and probably also perceived their immediate environment to be richer, so could afford to respond by flying away or stopping feeding sooner than birds in poorer condition which were not provisioned with food (Gill *et al*., 2001). The results of this study do not mean that short-term behavioural evaluations have no value, only that behavioural responses to disturbance must be interpreted with caution. Short-term assessments conducted at specified intervals over periods of years can be useful in detecting, for example, behavioural change over time, and thereby, distinguishing among mechanisms for responses resembling habituation (Bejder *et al*., 2006).

In recent years, heart rate has been used to estimate the energy expenditure of free-ranging animals under disturbed conditions (Green *et al*., 2005; Ellenberg *et al*., 2013). In Yellow-eyed penguin's (*Megadyptes antipodes*) the time needed to recover after natural disturbance events was minimal when compared to the long recovery times needed to recover from a human disturbance event (Ellenberg *et al*., 2013). Visitor-penguin was the primary determinant for predicting the heart rate responses of penguins, with individuals showing no sign of recovery as long as a person was within sight. The behaviour of the tourists was also important; a person who carefully moved around the colony provoked a greater heart rate response than a motionless human at the same distance did (Ellenberg *et al*., 2013). The results of this current study show that females which pupped further from the fence line spent more time engaged in resting behaviours and less time engaged in alert and vigilance responses. It may be that these seals also exhibit physiological responses to a reduced distance between themselves and visitors, similar to those observed in penguins, however much more research needs to be done to test whether this is indeed the case.

Sustained exposure to stressors will cause prolonged elevated glucocorticoid concentrations which are known to be physiologically damaging to individuals and have been to known to result in higher susceptibility to disease, reduced fertility and lower life expectancy (Wingfield *et al*., 1997; Ellenberg *et al*., 2007). If a large proportion of

the visited population is exposed to such impacts from disturbance events, there is a possibility that the conservation status of the affected population or even species could be put at risk (Lusseau *et al*., 2006). Different populations and individuals within a population will exhibit different levels of behavioural and physiological habituation to certain kinds of human disturbance, meaning management actions will need to be tailored for each unique situation (Cassini *et al*., 2004; Bejder *et al*., 2006; Tracey and Flemming, 2007).

4.4.2 Possible extensions to the current research

This study aimed to identify the behavioural responses of females to anthropogenic disturbance stimuli. The study was designed to act as a springboard for future research into the identification of any impacts that disturbances may have on the breeding behaviour of female grey seals and any future impacts that these responses may have on pup development and survival. A simple extension to the current study, and one which could assist in conclusively determining whether the seals at Donna Nook are indeed habituated to various forms of anthropogenic disturbances would be to repeat the same study over a number of breeding seasons. If responses to disturbances continue to be minimal or decline further over subsequent breeding seasons, the mechanism of habituation would be supported.

This is only the fourth known study of grey seals to be carried out at the Donna Nook site in which females were individually identifiable by photo ID. The production of this photo-catalogue would allow long term records to be built up over a number of breeding seasons, enabling data on the age and experience of females at the site to be built up. By tracking the same female over a number of years it would not only be possible to track the behaviour of her but also follow the development of her pups through the juvenile stage and into adulthood if there are high return rates to the colony and the pups are individually identifiable and/or marked/tagged. By combining data collection on females and their pups, it would be possible to determine whether the responsiveness of females to disturbance events is something which is passed on to their pup.

A further extension to this study could look at the effects that disturbances have on weaned pups; both while they are still on land, and also once they have gone off to sea. It is commonly known that when a group of weaned pups occur on a particular colony they will often form aggregations around areas where adult density is low and

survive off their blubber layer which they have built up during lactation (Hewer, 1974; Twiss *et al.,* 2001). It is not unusual for weaned pups to stay on the colony for several weeks after their mothers have gone back to sea (Fedak and Anderson, 1982). Although, as of when this study was published there has been no research into the effect that anthropogenic disturbances have on weaned pups on breeding colonies, one can hypothesise that where disturbances cause a behavioural or physiological response to weaned pups, there could be an associated impact on the chances of survival of that weaned pup. A reduction in the survival rate of weaned pups may be noted where weaned pups travel to the seal before they are ready or in instances where they waste a lot of energy whilst on the colony responding to disturbance stimuli.

One of the most exciting and interesting extensions to this study comes from identifying the impacts that long term disturbances may have on pup development to adulthood at the site. Behavioural observations used in this study could be combined with weight measurements and milk transfer efficiency from both mother and pup throughout rearing. Weight and energy transfer measures would provide an opportunity for comparison of maternal behaviour and pup weaning condition under differentiating disturbance conditions. Molecular analysis, focussing on the analysis of stress markers such as glucocorticoid measures would in addition allow us to observe any metabolic implications that disturbances at the site may be having on the stress responses of the females at the site, in the absence of any behavioural responses.

During this discussion, vigilance behaviours have been identified as a key indicator of a response to a disturbance event. Observations made during the current study suggest that mother-pup interactions and resting behaviours, too, appear to be affected by some forms of anthropogenic disturbances. An extension to this would be to produce an in-depth timeline of how seals respond to disturbance events in the first hour after a disturbance, as this has been previously shown to be when the most intense behavioural reactions to disturbances occur (Kovacs and Innes, 1990).

As alluded to earlier in this thesis, disturbances are factors of most ecosystems and have the potential to affect every dimension of a bionetwork, and can temporal dimensions (Fraterrigo and Rusak, 2008). The majority of studies which have investigated the impacts that's anthropogenic disturbances have on an individual/ population have chiefly looked at the effect of disturbances during daylight hours, however many disturbance sources are still present during the hours of darkness. Since aircraft fly-overs and key visitor attendance hours at the Donna Nook site are

usually between the hours of 9:00am until 5:00pm, examining the difference in behavioural protocols between these hours and overnight may provide some further insight into how disturbances affect behaviour as well as giving a greater knowledge into pinnipeds night-time behaviours on land (Allison and Destefano, 2006). Night vision equipment has seen great development over the past few decades, with costs of the equipment declining over the past decade as devices become more obtainable to the masses (Allison and Destefano, 2006). Although readily obtainable using night vision equipment to record animal behaviour would still come with its problems (Havens and Priest, 1995). Discerning fine scale behaviours such as pup checks may prove problematical as the resolution and detail on night vision equipment is still lacking. This being said with proper trials and grouping of behaviours into broad scale categories such as those used in this study, the use of night vision equipment to study the effects of disturbance on individual behaviour in the hours of darkness may still be achievable.

The use of satellite telemetry, whilst perhaps costly and technically challenging (James, 2013), could prove to be enormously informative. Pups could be tagged, after their initial lanugo moult, to provide information on their survival and behaviour during the following year (until their next moult). This would provide us with some information about how the survival rates of pups may alter in response to varying disturbance levels at the site. Tagging can take two forms, satellite telemetry (giving detail of an individual's position using a GPS system) or sensor networks (providing the same information as well as contacts made and social networks formed between tagged individuals)(Vincent *et al*., 2002; Lindgren *et al*.,2008). Tagging could provide invaluable information about the exploratory tendencies of known pups, as well as how individuals may respond to disturbance events outside of the breeding season.

5. CONCLUSION

The current study provides evidence to suggest that grey seal mothers show a very limited response to anthropogenic disturbance stimuli, possibly indicating that seals may be able to habituate to regular forms of human disturbance. Despite visitor numbers in the tens of thousands, there was little evidence to suggest any difference in maternal time budgets between focals with and those without various forms of anthropogenic disturbance stimuli. Responses to pedestrian disturbances were more frequent and intense than those behavioural disturbances to aerial disturbances. Expected behavioural changes in response to disturbance events such as increases in alert behaviours and a corresponding decrease in resting behaviours were observed amongst mothers at Donna Nook; however these changes were minimal and only present in univariate analysis, and were excluded as being relevant from more complex GLMMs. Behavioural responses to natural disturbance events, particular intraspecific aggressive encounters appeared to be more widespread that those associated with anthropogenic disturbance events. A combination of factors including public access restrictions enforced both by the fence and the dedicated efforts of the team of wardens at the site have prevented unnecessary maternal vigilance behaviour with a decreased in the behavioural responses of females to regular disturbance events. In addition to this, the study has confirmed the presence of CIDs in vigilance behaviours across disturbance context. The presence of CIDs in key behaviours indicative of a disturbance response means that individual personalities will need to be factored into future studies on disturbances in grey seals along with other key covariates (e.g. day of season, location, and pup sex).

The location of the females at the public site appeared to affect, the females responses to disturbance events. Females on the site close to the car park spent significantly less time engaged in alert, vigilance and threatening behaviours than those who reared their pups close to the RAF site. It is possible that the presence of disturbances at the public site, combined with the accessibility of the nearby, relatively undisturbed RAF site, places a selective pressure on individuals hauling out onto the colony with relation to pupping site. If an individual had a predisposed low tolerance to disturbance, pupping and breeding attempts on the public stretch of the colony may be relatively unsuccessful. Success may increase simply by selecting a pupping spot on the less disturbed RAF site. Those individuals with a higher tolerance towards disturbance may find adequate pupping and breeding success on the public site. This would explain the

dissimilarities in maternal behaviour between the two sites (James, 2013), as individuals on the RAF site would not experience the same levels of pedestrian disturbance as people tend to remain close to the public car park part of the path, and those on the public site show higher tolerance and, as a result, minimal if any change in behaviour.

Although it could not be included in the main models, there is some evidence from univariate analyses that indicates pup sex may have an effect of female responses to disturbance events. If this is indeed the case, pup sex will need to be taken into consideration in future studies. The results of this study provide support for the theory that females of male pups spend more time engaged in vigilance behaviours in the presence of anthropogenic disturbances than mothers of female pups. This supports previous evidence put forward by Kovacs, (1987) study on North Rona, where it was observed that mothers of male pups spent more time with their pup, and more time engaged in alert and defensive behaviours than mothers of females pups on the same island (Kovacs, 1987).

The most intriguing and exciting conclusion of this study is the preliminary evidence found for the existence of a diminished response to disturbance events in female grey seals during the breeding season. Whether this is indeed as a result of habituation or as a result of a focus on breeding related behaviours and a repression of the response to disturbance events, only future analysis of this subject will determine. Investigations into the behaviours of the same females over subsequent breeding seasons would be necessary to further support this idea that females at the Donna Nook site may have habituated to the frequent presence of anthropogenic disturbance during the breeding season. The results of this study suggest that the management strategies employed at the site appear to be successful; in so much that the disturbances attributed to human presence at the site do not appear to affect mother-pup interaction or nursing behaviours, two behaviours which are commonly associated with the likelihood of survival for the pup. The only anthropogenic demographic which affected the behaviour of females were photographers; thus indicating that tighter controls on photographer behaviour/numbers at the site may be needed. The results of this study, along with the continued rise in pup numbers at the site year on year speak positively for the future of the Donna Nook breeding colony. Nevertheless, the continued success may well be dependent on the continued restriction of the public, enforced by the warden team and the fence line. This will ensure that human disturbances remains as unobtrusive as possible and that further expansion of the colony can occur in the future.

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